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# SUMMER RESEARCH PROGRAM -- 1995

# GRADUATE STUDENT RESEARCH PROGRAM FINAL REPORTS

## VOLUME 12A
## ARMSTRONG LABORATORY


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Washington, D.C.

December 1995

## PREFACE

Reports in this volume are numbered consecutively beginning with number 1. Each report is paginated with the report number followed by consecutive page numbers, e.g., 1-1, 1-2, 1-3; 2-1, 2-2, 2-3.

This document is one of a set of 16 volumes describing the 1995 AFOSR Summer Research Program. The following volumes comprise the set:

| VOLUME | TITLE |
|---|---|
| 1 | Program Management Report |
| | ***Summer Faculty Research Program (SFRP) Reports*** |
| 2A & 2B | Armstrong Laboratory |
| 3A & 3B | Phillips Laboratory |
| 4 | Rome Laboratory |
| 5A, 5B, & 5C | Wright Laboratory |
| 6A & 6B | Arnold Engineering Development Center, Wilford Hall Medical Center and Air Logistics Centers |
| | ***Graduate Student Research Program (GSRP) Reports*** |
| 7A & 7B | Armstrong Laboratory |
| 8 | Phillips Laboratory |
| 9 | Rome Laboratory |
| 10A & 10B | Wright Laboratory |
| 11 | Arnold Engineering Development Center, Wilford Hall Medical Center and Air Logistics Centers |
| | ***High School Apprenticeship Program (HSAP) Reports*** |
| 12A & 12B | Armstrong Laboratory |
| 13 | Phillips Laboratory |
| 14 | Rome Laboratory |
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# A STUDY OF THE POSSIBLE ANTIFUNGAL AND ANTIMICROBIAL PROPERTIES OF THE COMMON GREENBRIER

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
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and

Armstrong Laboratory

August 1995

# A STUDY OF THE POSSIBLE ANTIFUNGAL AND ANTIMICROBIAL PROPERTIES OF THE COMMON GREENBRIER

Nicole M. Adams
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Abstract

Based on field observations, it appeared that *Smilax rotundifolia* or Common greenbrier had no natural predators. It was suggested that this plant might exhibit antimicrobial or antifungal properties. Several other plants in the *Smilax* genus demonstrate antifungal properties. The following experiments were designed to test the plant extracts for these properties. The first set of experiments were designed to examine the effects of plant proteins on the growth of *Pseudomonas aeruginosa* and yeast cells. To prevent denaturing of proteins, a non-boiling physical extraction process was used. The plant extract was tested on agar plates and then in liquid cultures. After receiving literature which contained a procedure for extraction of *Smilax* compounds which inhibited fungal growth, the extraction process was modified to include a boiling process, an evaporation process, a filtering process, and a phase to monitor the long term results. The results show that the tuber or main root of the plant has some compound(s) that inhibits the growth of yeast cells.

# A STUDY OF THE POSSIBLE ANTIFUNGAL AND ANTIMICROBIAL PROPERTIES OF THE COMMON GREENBRIER

Nicole M. Adams

## Introduction

The plant *Smilax rotundifolia*, also known as Common greenbrier, grows throughout the East, from Nova Scotia to Florida. Common greenbrier is a climbing vine which produces berries and is a food source for wild game and poultry (1). Based on field observations, it appeared that *Smilax rotundifolia* had no natural predators. It was suggested that this plant might exhibit antimicrobial or antifungal properties. Several other plants in the *Smilax* genus demonstrate antifungal properties. The following experiments were designed to test the plant extracts for these properties.

## Methodology

### Plant Material

Initially, plant material was obtained from several locations and by different individuals, this resulted in the plant material being a few days old. The secondary roots of this sample were tested when they were two days old. The tuber of this sample was five days old when tested. The leaves were two days old when tested. After locating an area where Common greenbrier grew abundantly, we obtained supplies the same day the extraction process occurred.

### Extraction Techniques

#### Non-boiling

The first set of experiments were designed to examine the effects of plant proteins on growth of *Pseudomonas aeruginosa* and yeast cells. To prevent denaturing of proteins, a non-boiling physical extraction process was employed. The first portion of the plant tested was the secondary roots. After being clipped into small pieces about 2 cms long, the secondary roots were placed in a funnel on top of Whatman Filter paper #42 11 cms and washed with milli-Q water. They were then dried and 1 g of root material was weighed out. The secondary root was cut into 1/2 square cms and ground up for ten minutes using a mortar and pestle. Ten ml of sterile water was

added, and the roots was ground up for an additional ten minutes. The tuber, which is the main root, was also tested using this extraction method. When the experiment was modified to a liquid stage, the extraction process remained the same except 2 grams of leaves were weighed out and 20 ml of sterile water was used in the grinding process.

Boiling

After obtaining literature which contained a procedure for extraction of *Smilax* compounds which inhibited fungal growth, the experiment was modified once more (2).

The tuber was washed, thoroughly dried, and 40 grams were weighed out. While the tuber was cut into small pieces using a Waring blender, 200 ml of sterile water was added. The solution was boiled for five minutes then placed on a rotary evaporator for concentrating extract. The concentrated solution was placed in polyvinyl sterile tubes and spun in the centrifuge to separate the liquid from the solid matter. The first layer of liquid was pre-filtered using a 47 mm Whatman Microfibre #934-AH filter. The pre-filtered solution was then filtered through a .22 um Millipore filter.

**Antifungal Screen**

Disk Method

The plant extracts were tested with brewer's yeast, *Saccharomyces cerevisiae*, and *Pseudomonas aeruginosa* (PAO-1), a bacteria. Plates of tryptic soy agar (TSA) and peptone glucose acid agar (PGAA) were prepared in order to grow the bacteria and the yeast. Both PAO-1 and Fleischmann's Yeast was cultured on agar plates to obtain individual colonies. Six disks of Nalgene 200-4020 membrane filter non-sterile .2 um and chromatography paper (Whatman No. 3) were soaked for ten minutes in the extract solution and then blotted on chromatography paper (Whatman No. 3) to remove extra solution. The controls were six more disk of each type of filter paper that were soaked in sterile milli -Q water and the excess water was removed by blotting. The yeast and PAO-1 cells were resuspend in 5 ml of phosphate buffer. An absorbency reading of the cells was obtained by using Beckman DU 65 spectrophotometer set at 600 nm. The desired reading was approximately .50. 0.1 ml of resuspended yeast cells were placed on each of three PGAA plates and 0.1 ml of PAO-1 cells on each of three TSA plates, then the cells were spread over the surface evenly of the agar plates. Two disks of both Nalgene 200-4020 membrane

filter non-sterile .2 um and Chromatography paper (Whatman No.3) that were soaked in extract solution were placed on each agar plate. Two control disks of both types of filter paper were also placed on the agar plate along with the other disks. The plates containing the disks were placed in a 25°C incubator and measurements of the zones of inhibition around the disks were taken every 12 hours for 48 hours.

In order to ensure protein was being extracted from the plant material, we conducted protein assays on extracted material. The dilutions of the plant extract tested for protein was 1:2, 1:10, 1:50, and 1:100. The absorbency readings were taken on Beckman DU 65 spectrophotometer set at 562 nm. A standard curve using Bovine Serium Albumin (BSA) was performed by standard methods.

Liquid Cultures

Because the amount of extract on the disks could not be controlled, the experiment was modified to use liquid cultures, peptone glucose acid broth(PGAB) and tryptic soy broth(TSB), to grow the yeast and PAO-1 cells. PGAB and TSB were each placed in nine flasks in increment of 25 ml. The extract solution was added to the broths in increments of 250, 500, and 1000 ul. Each measurement had two separate flasks per broth. Sterile water was substituted for the extract solution as controls. After taking an absorbency reading of the yeast and PAO-1 cells, which had been growing overnight, 1 ml was added to each flask. An absorbency reading on the spectrophotometer was conducted every two hours for 24 hours starting at zero hours. After the 24 hour reading, the liquid cultures tested for contamination.

Following the boiling extraction procedure, 25 ml of TSB and PGAB was added to nine flasks and extract solution of 100 ul, 250 ul, and 500 ul were added to the broths. The amount of extracted material added was reduced due to the fact that the present solution has a higher concentration. To improve accuracy we set-up two separate flasks for each amount of extracted material. Control flasks contained sterile water instead of plant extract. After taking an absorbency reading of the yeast and PAO-1 cells, which had been growing over night, 500 ul was added to each flask. An absorbency reading on the spectrophotometer was conducted every two hours for 24 hours starting at zero hours. After the 24 hour reading, the liquid cultures were tested for contamination. In order to examine the long term effect of cells exposed to plant extracts a second phase was undertaken where cells exposed to the extract solution were placed in new media with fresh plant extract. More PGAB and TSB were each placed in nine

flasks in increments of 25 ml. Fresh extract solution was added to the broths in increments of 100, 250, and 500 ul. Each measurement had two separate flasks per broth. Sterile water was substituted for the extract solution in order to create controls. Yeast and PAO-1 cells (0.5 ml) were taken from the first set of flasks and placed in the new flasks. An absorbency reading on the spectrophotometer was conducted every two hours for 24 hours starting at zero hours. Following the 24 hour reading, the liquid cultures were tested for contamination. Along with the tuber, the berries and leaves were tested in this same manner.

## Results

### Disk Method

There was no zones of inhibition around any of the discs which were soaked in extract. The following graphs show the results of all the liquid cultures.

### Non-boiling, Liquid Cultures

The first set of graphs are the results of the non-boiling process of the leaves. Both the Yeast and PAO-1 graphs show little difference between the growth of the cells exposed to water and the cells exposed to the extract solution. The only contaminated liquid culture was the PGAB flasks that contained 250 ul of extract.

### Boiling, Liquid Cultures

After the boiling process took place, phase one graphs of the leaf extraction for both yeast and PAO-1 also showed little or no sign of inhibition of the cell growth. The liquid cultures that were contaminated were one of the 500 ul PGAB flasks containing extract solution and the PGAB control containing 250 ul of water. Phase two graphs of the leaf extraction had similar results to Phase one. There was no sign of contamination in any of the liquid cultures. Phase one graphs of the berry extraction for both Yeast and PAO-1 showed little or no sign of inhibition of the cell growth. There was no sign of contamination on any of the liquid cultures. Phase two graph of the berry extraction for PAO-1 had similar results to phase one but the graph for Yeast showed that the grow of the cells exposed to extract was higher then that of the cells exposed to water. The PGAB flasks that contained contamination were both flasks with the increment of 500 ul, one with the increment of 250 ul, and all control flasks. The phase one graph of the root extraction for PAO-1 showed little or no sign of inhibition of the cell growth. The phase one graph of the root extraction for yeast was the only one that showed a significant amount of

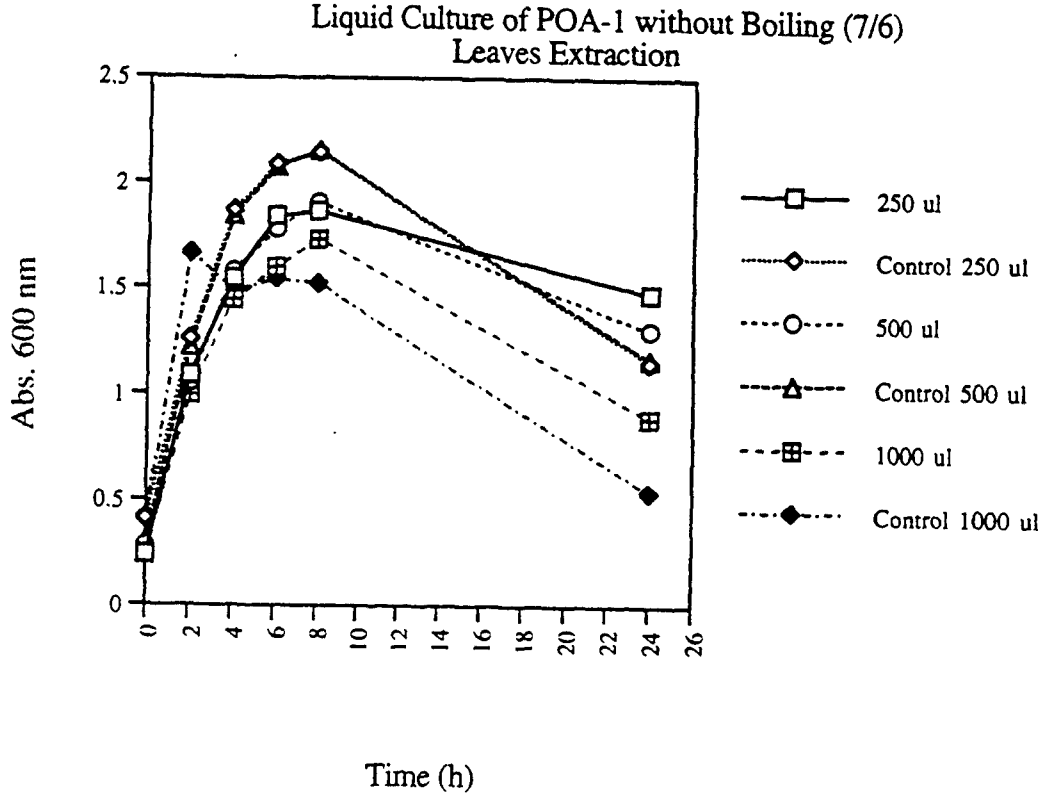
inhibition of the cell growth. Phase two graphs of the root extraction had similar results to phase one. The liquid cultures that were contaminated were both of the PGAB flasks that contained 250 ul of extract and a PGAB flask that contained 500 ul of extract.

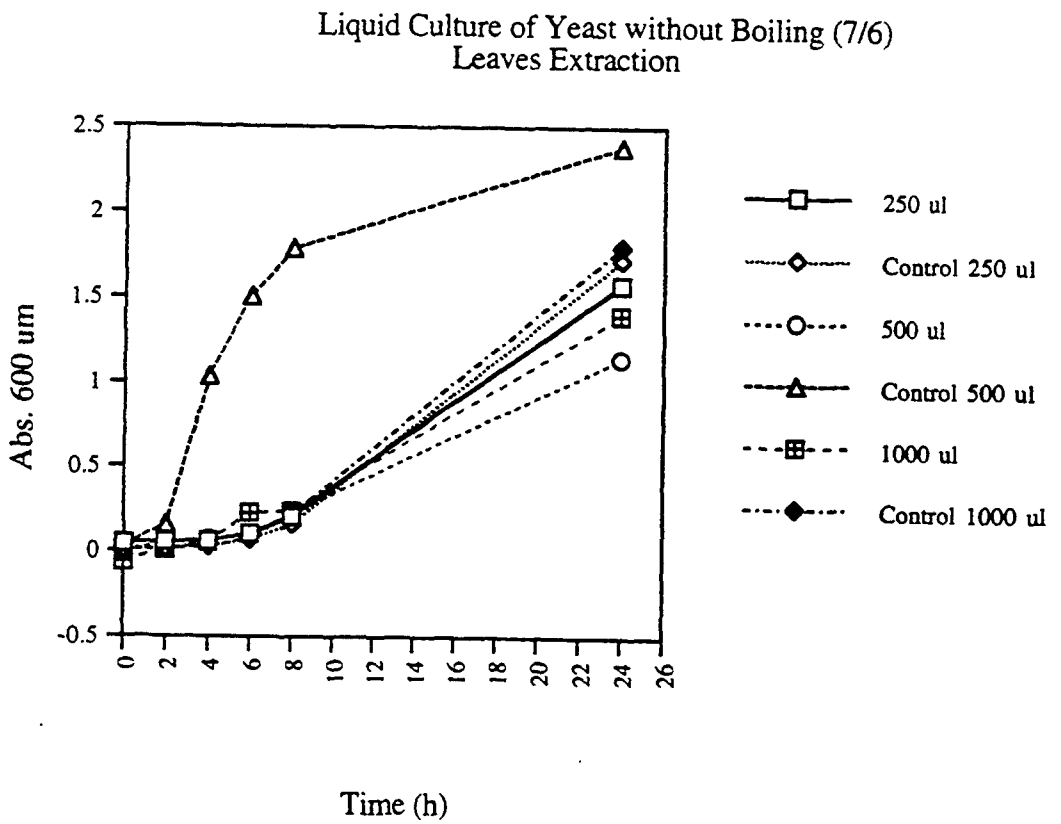
## Conclusions

Based on the data gathered, one might conclude that Smilax rotundifolia contains an antifungal property in the tuber portion of its roots. There is a possibility that the antifungal compound(s) in the root are used up over time, which would explain why the exposed cell growth rose to the same level as that of the controls at the end of the 24 hours.

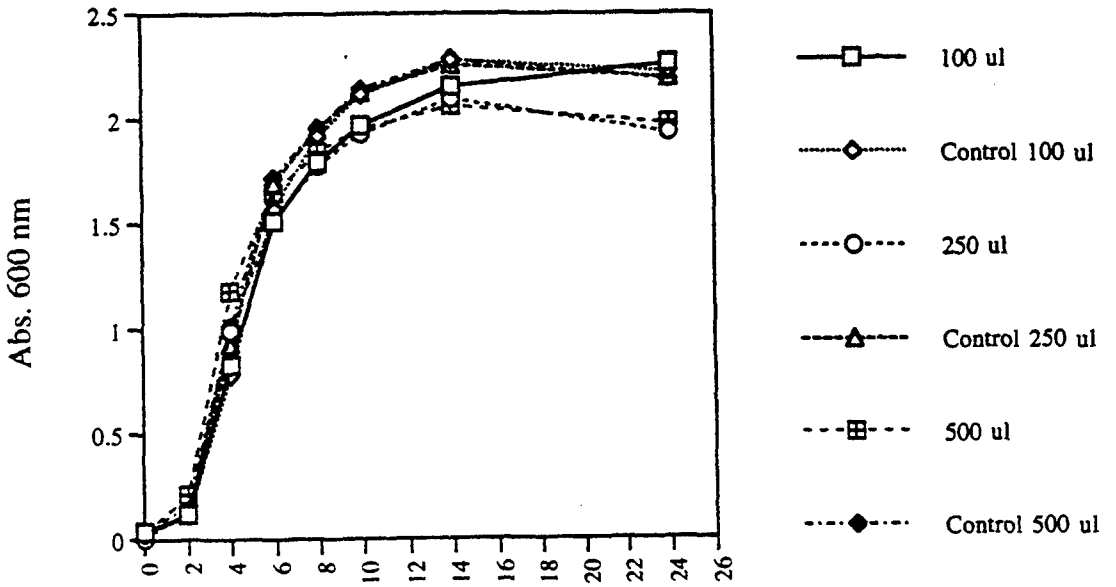
## References


1. Smith, Robert L. *Greenbriers.* W. Virginia University Morgantown. pp. 54-58
2. Caceres, A., Giron, M.A., Logemann, H., and Lopez, B.R. (1990) Plants used in Guatemala for the Treatment of Dermatophytic Infections. 1. Screening for Antimycotic Activity of 44 Plant Extracts. *Journal of Ethnopharmacology* 31, 263-276.


Liquid Culture of POA-1 without Boiling (7/6)
Leaves Extraction
Abs. 600 nm
Time (h)
250 ul
Control 250 ul
500 ul
Control 500 ul
1000 ul
Control 1000 ul

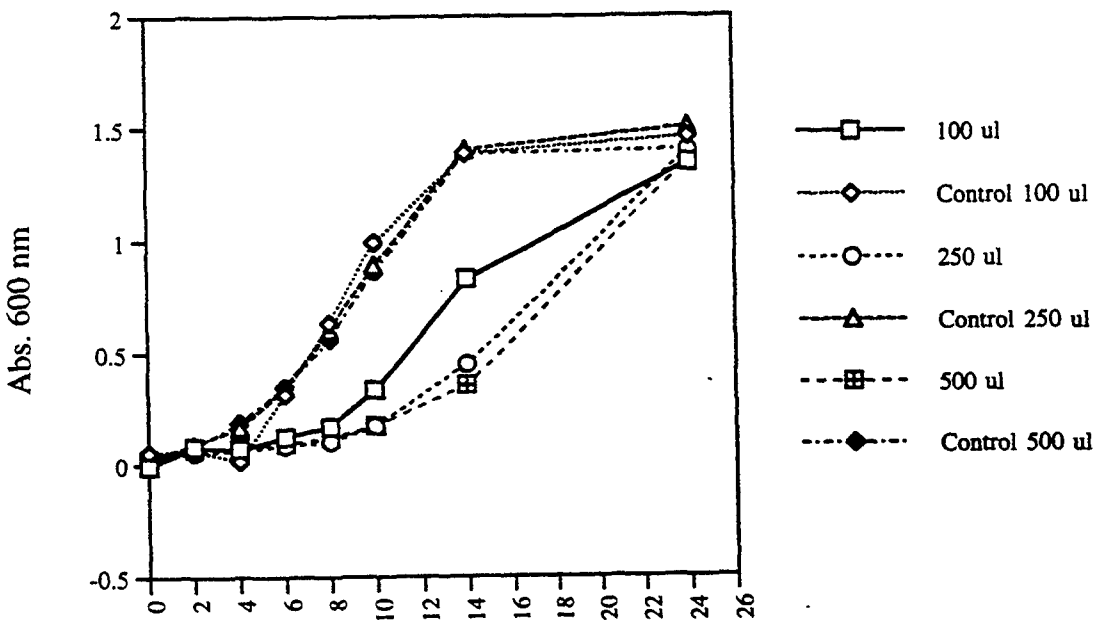

Liquid Culture of Yeast without Boiling (7/6)
Leaves Extraction
Abs. 600 um
Time (h)
250 ul
Control 250 ul
500 ul
Control 500 ul
1000 ul
Control 1000 ul

Liquid Culture of PAO-1 Phase 1 (7/18)
Root Extraction



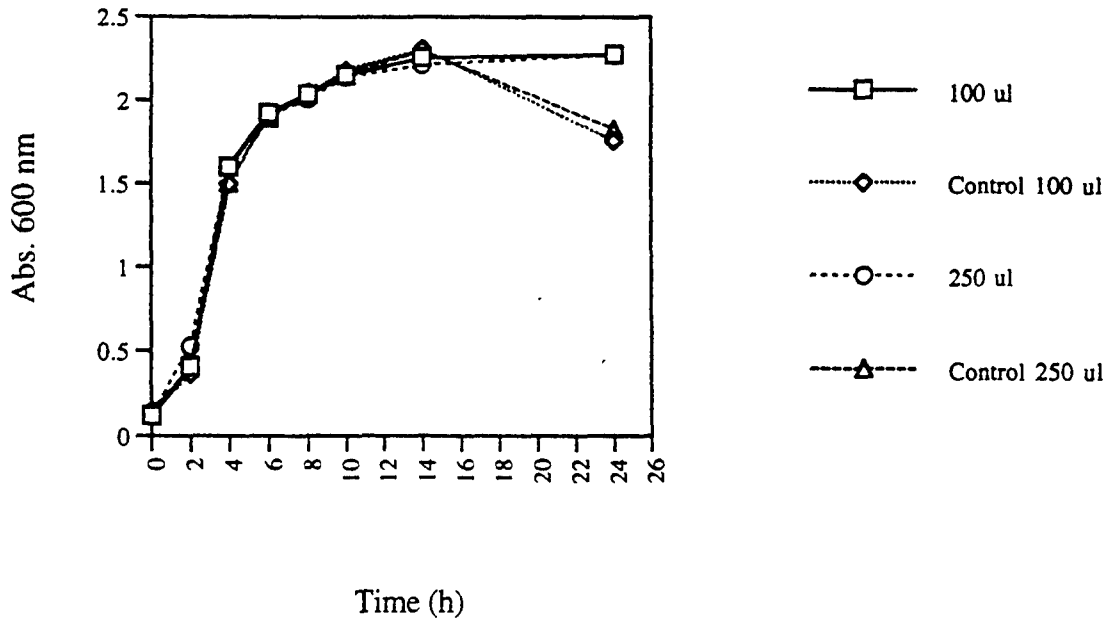

Time (h)

Liquid Culture of Yeast Phase 1 (7/17)
Root Extraction

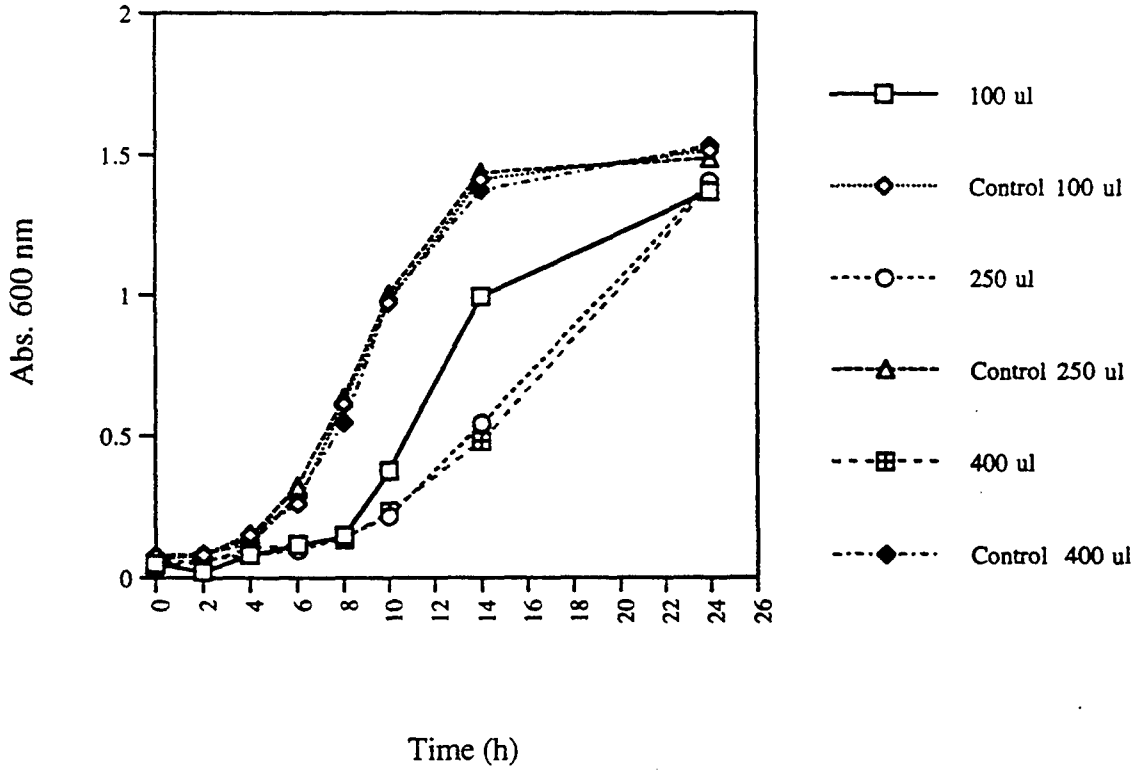



Time (h)

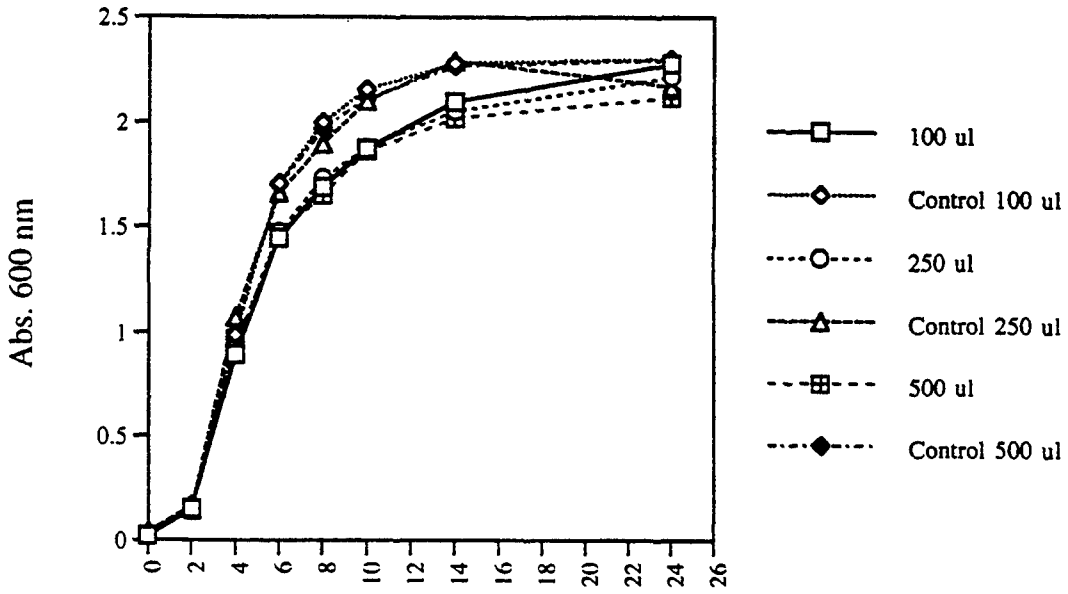
Liquid Culure of PAO-1 Phase 2 (7/19)
Root Extraction




Liquid Culture of Yeast Phase 2 (7/18)
Root Extraction



Liquid Culture of PAO-1 Phase 1 (7/20)
Leaf Extraction

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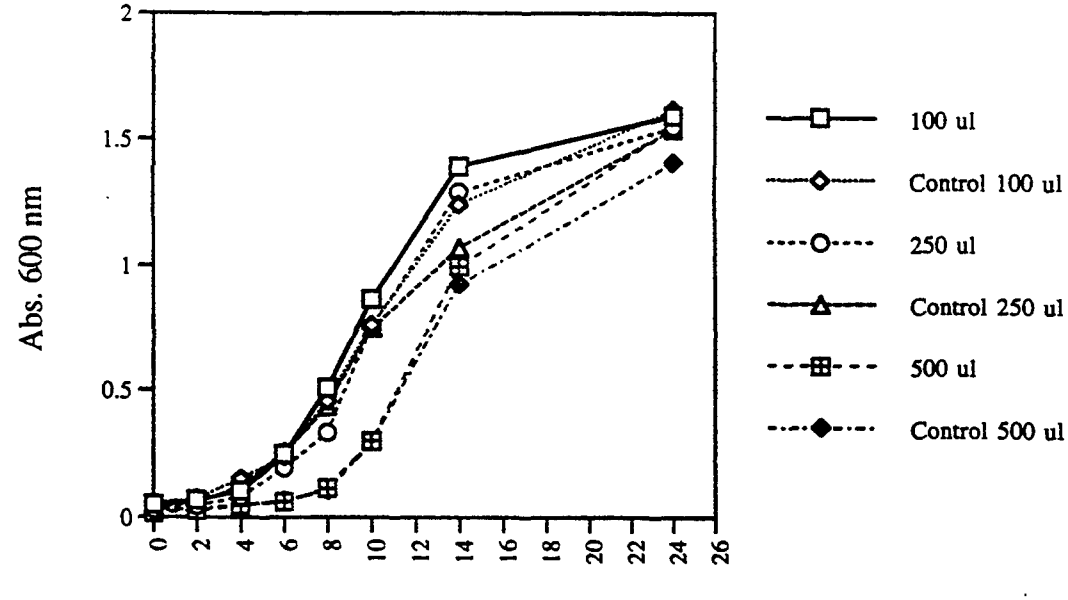
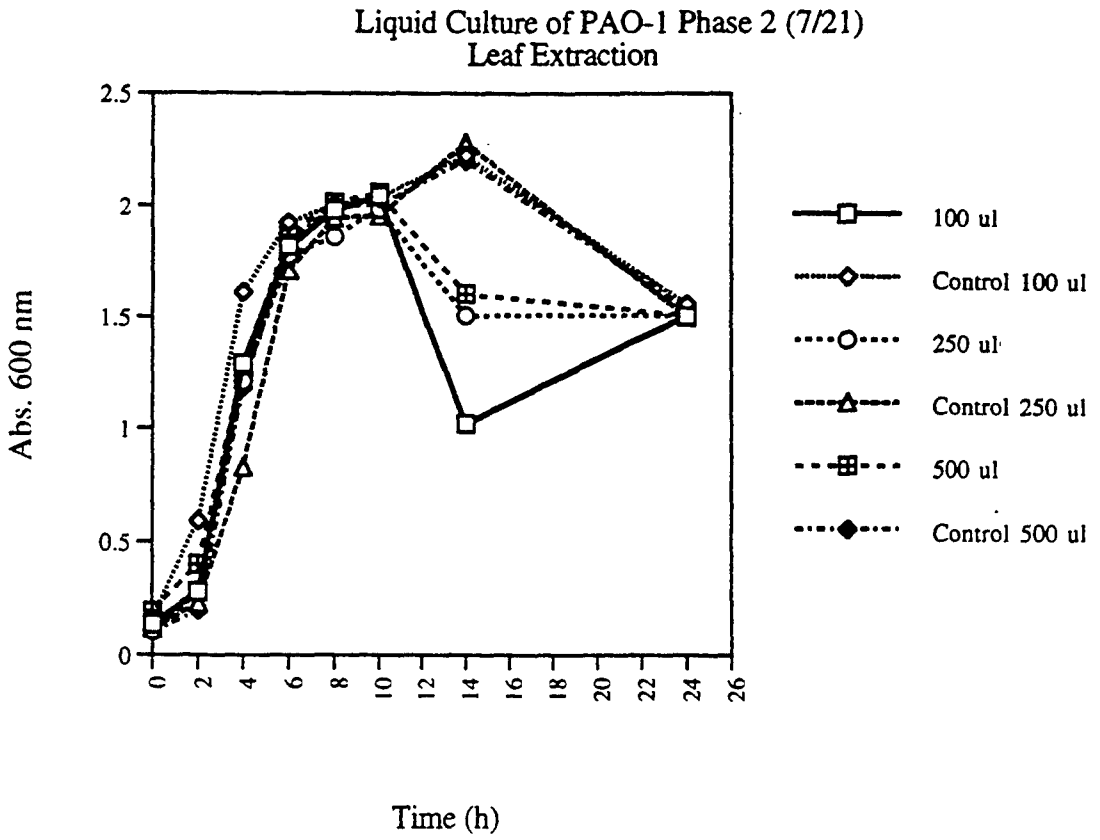
Leaf Extraction

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Time (h)
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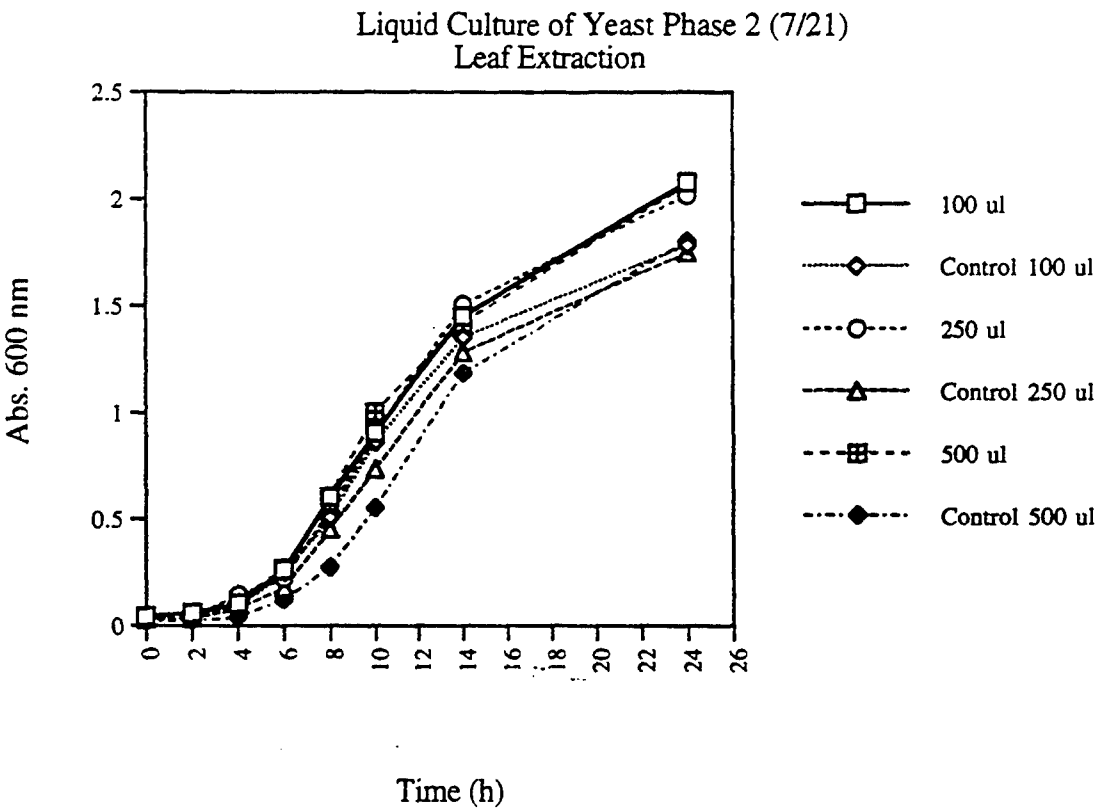
Control 100 ul
250 ul
Control 250 ul
500 ul
Control 500 ul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Abs. 600 nm
Time (h)
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Control 100 ul
250 ul
Control 250 ul
500 ul
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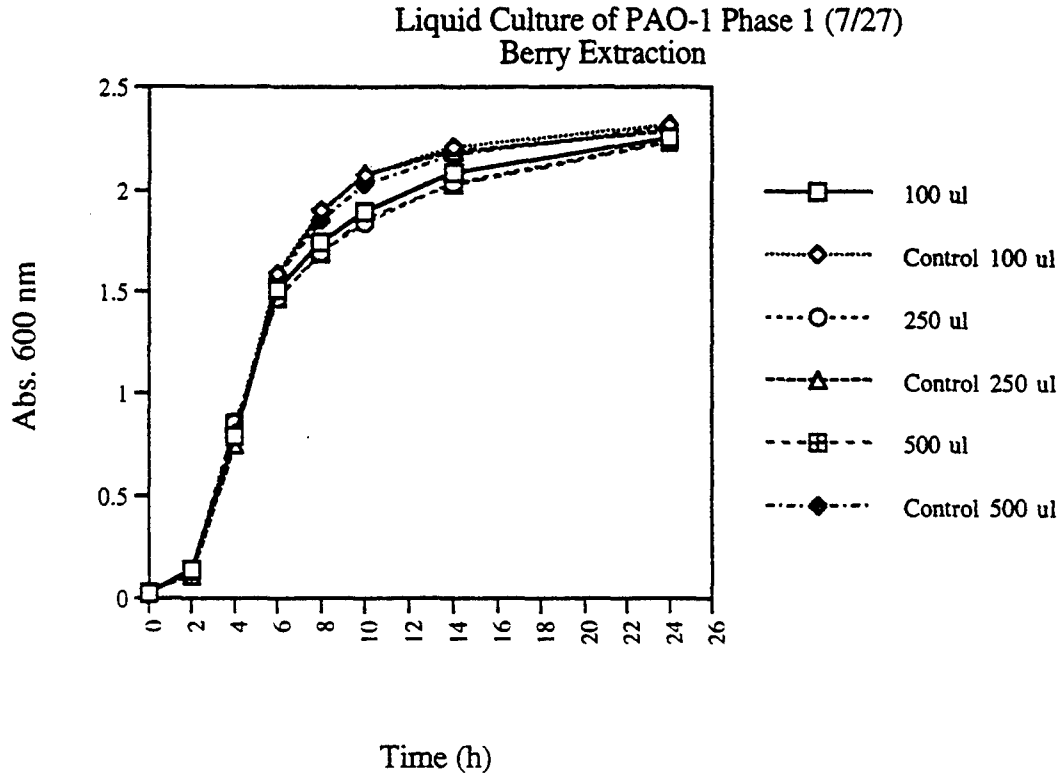
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Abs. 600 nm
Time (h)
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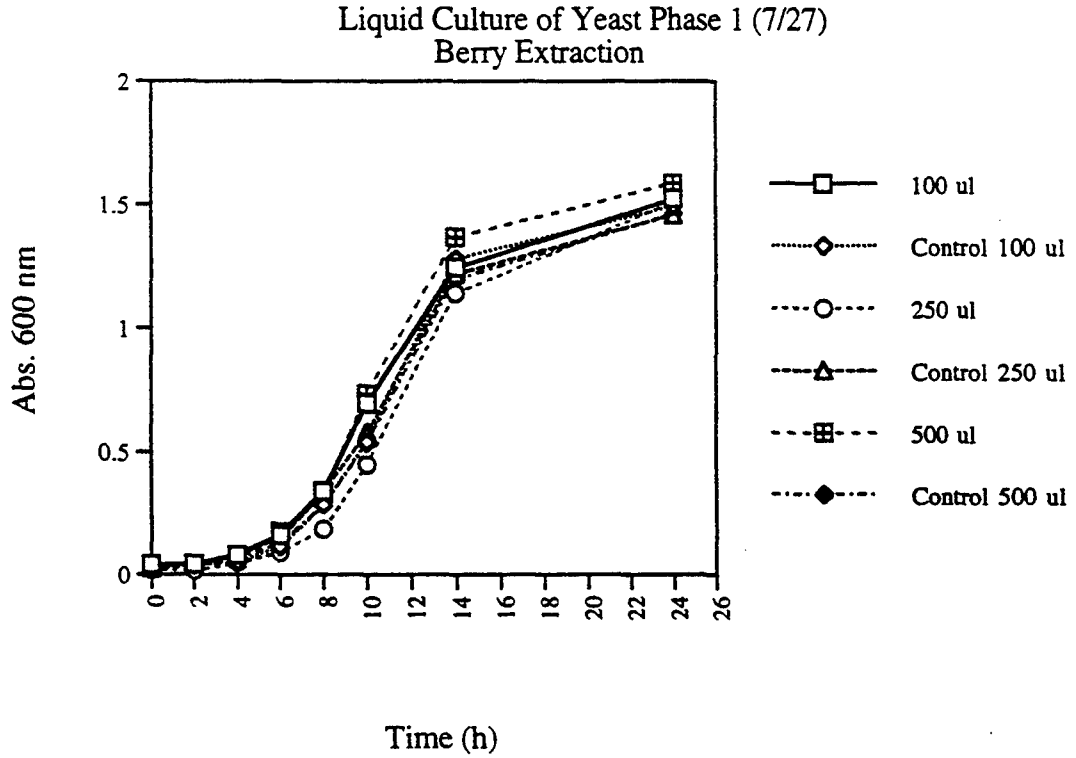
Control 250 ul
500 ul
Control 500 ul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Berry Extraction
Abs. 600 nm
Time (h)
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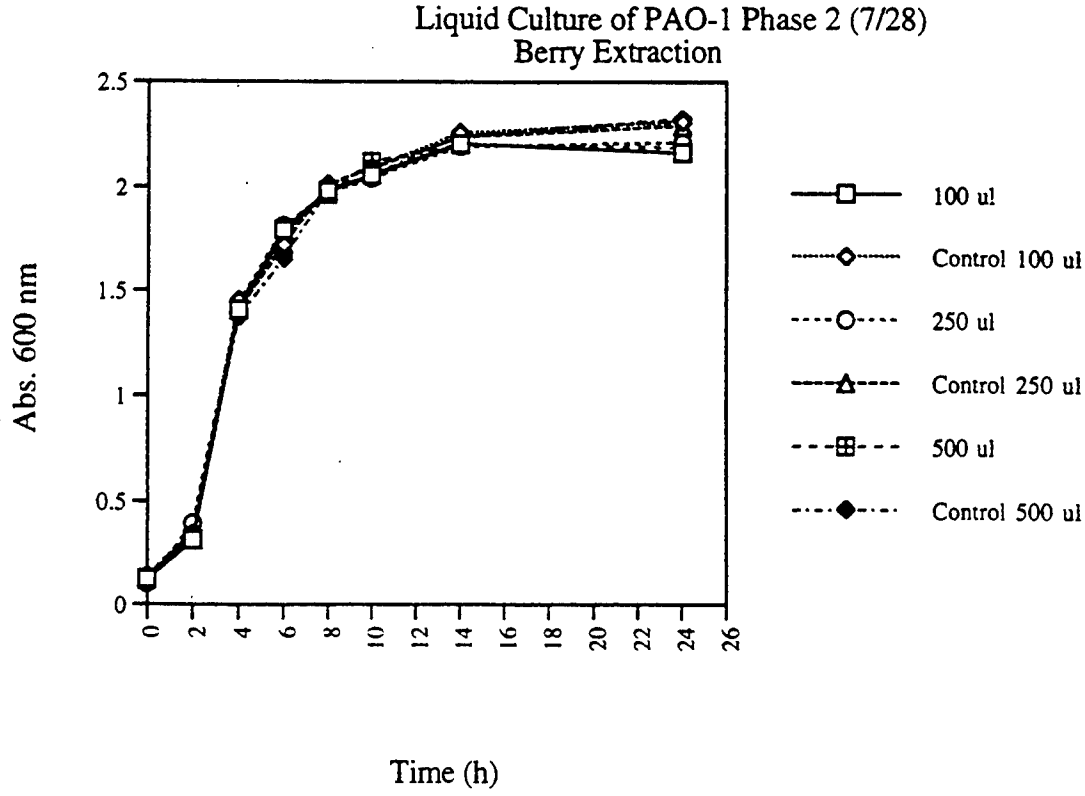
Control 100 ul
250 ul
Control 250 ul
500 ul
Control 500 ul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Time (h)
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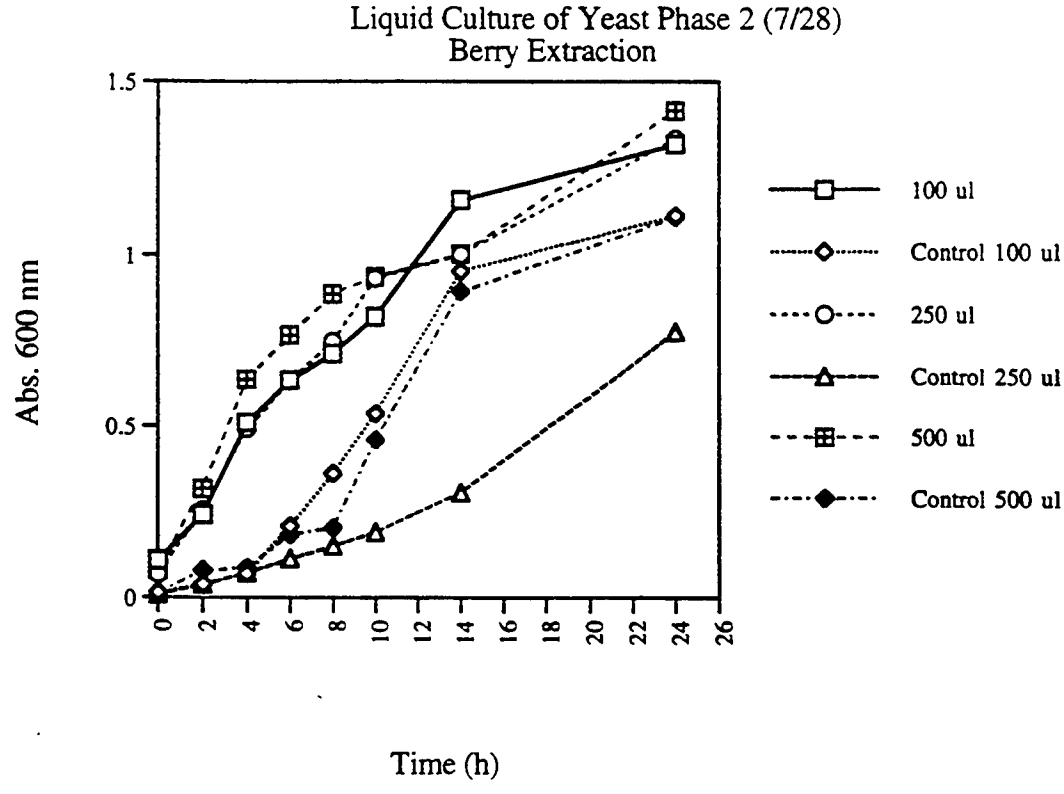
Control 100 ul
250 ul
Control 250 ul
500 ul
Control 500 ul


Liquid Culture of Yeast Phase 2 (7/28)
Berry Extraction
Abs. 600 nm
Time (h)
100 ul
Control 100 ul
250 ul
Control 250 ul
500 ul
Control 500 ul

# PUBLISHING ARMSTRONG LABORATORY TECHNICAL DOCUMENTS ON THE WORLD WIDE WEB

Anita Anderson

Judson High School
9142 FM 78
Converse, TX 78109

Final Report for:
High School Apprenticeship Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
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and

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August 1995

# PUBLISHING ARMSTRONG LABORATORY TECHNICAL DOCUMENTS ON THE WORLD WIDE WEB

Anita Anderson
Judson High School

Abstract

The World Wide Web is an effective, low-cost platform for information presentation. Creating and posting Technical Papers and Technical Reports on the Web allows Air Force researchers to reach a wider audience for a lower cost than printing and distributing paper documents. Because many Air Force Technical Papers and Technical Reports are authored in *Microsoft Word*, an easy to follow guidebook for changing such documents into HyperText Markup Language is needed. The necessary steps to change *Microsoft Word* documents into HyperText Markup Language are outlined with suggested readings for further information. Such methods were found to produce effective results.

# PUBLISHING ARMSTRONG LABORATORY TECHNICAL DOCUMENTS ON THE WORLD WIDE WEB

Anita Anderson
Judson High School

## INTRODUCTION

The World Wide Web is a rapidly expanding platform for information presentation. The concept behind the Web was developed by Tim Berners-Lee of the European Particle Physics Laboratory in 1989 (Hughes, 1994). Growth in the past year has been spurred largely by the introduction of easy to use browsers, which support the appealing multimedia presentations offered at many Web sites. Web documents are posted using HyperText Markup Language. The markup guides the browser in how sections of text should be displayed, but the browser's settings actually determine the physical appearance of a document.

As the number of Web sites continues to increase, the diversity of posted information also increases. The Web has become an appropriate means of publicizing organizational and research information. As part of the effort to provide the public with access to non-sensitive military information, the Air Force and its bases and laboratories have begun creating and maintaining sites on the World Wide Web. Such sites provide access to organizational information, project listings, personnel listings, and base information.

## DISCUSSION OF PROBLEM

In an age of decreased military spending, it has become important to reap the most benefit from available funds. The dual-use potential of research has become an important point in evaluating projects. Air Force researchers collaborate with their counterparts in universities and research corporations. Such collaborations are often carried out long-distance; arranging face to face meetings to present and exchange ideas and information is costly and time consuming. The use of a multimedia presentation platform such as the Web can greatly decrease costs while reaching a wider audience.

Scientists in Air Force Laboratories publish their findings in Technical Papers and Technical Reports. Such reports are printed on paper and receive limited distribution. The challenge is to find a way to meet the need for effective, long distance presentation of ideas in a timely and efficient manner.

METHODOLOGY

Because numerous Air Force technical papers and other documents for World Wide Web posting are in *Microsoft Word*, the standard word processing package for Armstrong Laboratory, this paper has been prepared as a guide to assist individuals in converting Word documents into effective HyperText Markup Language documents.

**System preparations**

1. Download and install *Microsoft Internet Assistant for Word* or another HTML tool program.
2. Download and install *L View* or another graphics program.

*To download a file:*

Create a new directory on your hard drive. If you are an authorized user of the Brooks Air Force Base server and the desired program is posted on the Brooks server, download from the Brooks server. (Detailed information on how to download is in Appendix A.) Otherwise, the material can be downloaded from the distributor. For program addresses, see Appendix B.

After downloading the file, change to your new directory in the File Manager and double click the file to uncompress it. Most files are self-extracting. "Refresh" the window in File Manager and complete any necessary installation or setup.

**Document preparations (using *Internet Assistant for Word*)**

1. Open the file to be changed to HyperText Markup Language.
2. When someone follows a link to a document, he or she will want to spend the least amount of time waiting for the document to appear on his or her screen. Several smaller documents make more effective use of HyperText format than one larger document. If the file is excessively large or contains numerous graphics, it is generally best to break it into several smaller files. Choose sensible places in which to break the file; for example, introduction, discussion, method, results, references, etc. Save each section of the document in its own file.
3. For each file, under the "File" menu, choose "Save As".
4. In the "Save File As Type" box, choose HyperText Markup Language (HTML) and click "OK". This creates a new file which will be in HTML. The new file will keep the same base filename, but the .doc extension will become .htm to indicate the file is in HyperText Markup Language. *Internet Assistant* will add HTML tags and display the new document.

At this point, there are no graphics in the document. Graphics must be added as separate files.

For each image, several items must be taken into consideration.

1. The larger and more complicated the image, the longer the delay before it appears on the screen. For this reason, it is often better to include only smaller versions of graphics in a document and link the smaller versions to the full-sized images. On the same note, images with millions of colors also take longer to load. Determine whether the millions of colors are worth the wait.
2. As discussed earlier, the reader's browser actually determines the appearance of a document. Browsers do not all support the same graphics file types. Graphics Interchange Format (GIF) files are widely supported. Joint Photographic Experts Group (JPEG) files are gaining support as browsers change. Currently, all graphics should be in one of these two formats. In deciding which file type to use, consider file size and the detail with which the image should be displayed. The compression ratio for JPEG files is determined at the time they are saved. By changing the compression ratio, the size of JPEG files can be controlled. Bear in mind, when decreasing the file size, the clarity of the image is sacrificed. For large images, however, JPEG is usually best.

After deciding the size and file format for each image, a file must be created for each. If the document's graphics are already saved as separate graphics files, the files only need to be saved in the appropriate file format (GIF or JPEG). This can be easily accomplished using the "Save As" or "Export" options in a graphics program. Graphics resources can be found in Appendix C.

If the graphics in the document are not separate files, the files must be created using a graphics program. "Maximize" the *Word* window size. Position the scroll bars so as much of the graphic as possible is showing. While holding the Alt key, press the Print Screen key. This copies the image from the screen onto the Clipboard. Some graphics programs allow direct "Paste" of a screen capture from the Clipboard, but *L View 3.1* does not. Open a program such as *Paintbrush* and maximize the size of the window. If necessary, remove the palette and toolbar. "Paste" the image. Save as a bitmap. In *L View*, open the saved bitmap. Crop as needed. In the "Save File As Type" box, choose GIF or JPEG. After the graphics files are saved, create smaller versions of graphics as appropriate by resizing the larger graphics and saving them under separate filenames.

After the graphics have been prepared, several changes must be made in the HTML files generated by *Internet Assistant*. Any questions arising about specific instructions in this section can be easily answered by examining some of the basic HTML references in Appendix D.

1. Open a text editor such as *Notepad* in Windows. A word processing program can be used if it can save files in ASCII format.
2. Open the first HTML file for the document.
3. In the <HEAD> element, there should be a <TITLE>. If there is none, add the document's title as shown: `<TITLE>Document's Title</TITLE>`.
4. After becoming familiar with HyperText Markup Language, files can be modified to increase readability. HTML references are listed in Appendix D. Consider adding or changing headings or text styles to emphasize various parts of the document.
5. Add the graphics. Instructions on adding graphics can be found in the HTML reference material. For in-line images (generally the smaller versions of the graphics), the following tag must be inserted in the file in the location the graphic should appear. `<IMG SRC="URL" ALT="alternate text">` Where *URL* is the path and name of the graphics file and *alternate text* is text which would appear only when the reader's browser is not set to view the image. URL example: If the graphics file is in a subdirectory, "images", and is named "overview.gif", the URL would be "images/overview.gif". If the image is a smaller version of another image, then clicking on the smaller image should link to the larger image. The in-line image tag would be surrounded by a HyperText Reference tag. Together, the tags would be `<A HREF="large graphic's URL"><IMG SRC="small graphic's URL" ALT="alternate text"></A>`
6. Repeat for each file in the document.
7. Link each file together. Use HyperText References to add *Previous*, *Next*, and possibly *Table of Contents* links at the bottom of each page. If the document is long, a table of contents should also be created with links to each section. To link to another file in the document, the following format is used. `<A HREF="filename">Section name</A>` Where *filename* is the name of the file with the section and *Section name* is the displayed text the reader will click to follow the link. The same tag applies for Previous, Next, and Table of Contents. For each, change *filename* to reflect the correct preceding and succeeding filenames. For links within the same file, the following format is used. `<A HREF="#anchor name">Section name</A> ...<A NAME="anchor name"></A>` Where *anchor name* is a name assigned to a given point in the document. *Section name* is the text the reader will click to follow the link. The second tag is located at the point in the document where the link should lead.

## RESULTS & CONCLUSIONS

Feedback from individuals who provided documents for Web preparation as well as others in the Human Resources Directorate was positive. HyperText Technical Reports and Papers are available any night or holiday. Additionally, the accessibility of the paper itself and links to resources and references result in time savings for the reader. The laboratory saves printing and mailing costs to reach a much wider audience than printed material could allow. Converting *Microsoft Word* documents to HTML is an easy process resulting in tangible savings for the laboratory.

## REFERENCES

Note: Addresses in the references and appendices were active as of the time of writing. Documents are often moved and may no longer be available at these addresses.

Hughes, Kevin. "Entering the World Wide Web: A Guide to Cyberspace". http://www.eit.com/web/www.guide/guide.04.html. May 1994.

Appendix A

How to Download Files

FTP to Brooks server

By opening FTP from the PC/TCP WinApps program group in Windows, you can connect to the Brooks server. If you have not previously saved a session with the Brooks server, you must establish a new session. Choose "New" from the "File" menu. Enter the following information:

Host name or address: ftp.brooks.af.mil

Username: anonymous

Password: guest

Choose "Connect". Save the session and name it Brooks.

On the left hand side should be listing of files on your computer's hard disk. The right hand side lists the files on the Brooks server. On the left hand side, change to the new directory you created. (The "[..]" can be clicked on to move higher in the directory structure until you reach your root directory.) Find the desired file(s) and copy. For example, to obtain *Internet Assistant*, choose "pubs" on the right hand side. In the "pubs" directory, choose "windows". In the "windows" directory, choose "htmltools". Select the "wordia.exe" file. The "Copy" button in the middle of the window should have arrows pointing towards your system (left). Copy. Close and exit your session.

FTP to another server

Follow the same procedure. Enter the appropriate host address. Companies normally state the appropriate username and password for anonymous file transfer protocol. The words anonymous, guest, password, and ftp are frequently used. For example, to get to Microsoft's ftp site, you might use "ftp.microsoft.com" as the hostname, "anonymous" as the username, and "guest" as the password.

Appendix B

Program Addresses

Internet Assistant for Word

*Internet Assistant* is available on the Brooks server in the pubs/windows/htmltools directory. The filename is wordia.exe. Exact downloading directions can be found at the end of this paper. *Internet Assistant* can also be obtained from Microsoft Corporation. Microsoft offers *Internet Assistant* over anonymous ftp at http://www.microsoft.com/MSOffice/Word/ia/default.htm.

GIF graphics programs

WinGif 1.4 or L View 3.1 for Windows is available at:

Merit Archive

gopher://gopher.archive.merit.edu:7055/11/msdos/windows/graphics/util/lview31.zip

Oak.oakland.edu

ftp://Oak.oakland.edu/SimTel/msdos

wuarchive.wustl.edu

ftp://wuarchive.wustl.edu/systems/ibmpc/msdos

archive.orst.edu

ftp://archive.orst.edu/pub/mirrors/simtel/msdos

Appendix C

Graphics Resources

GIF Resources

Yahoo directory of GIF resources

http://www.yahoo.com/Computers_and_Internet/Software/Data_Formats/GIF/

Transparent/Interlaced GIF resources

http://dragon.jpl.nasa.gov/~adam/transparent.html

Working GIFs

http://www.ozonline.com.au/cohort/howto/tgif/tgif.html#transparent

JPEG Resources

Yahoo directory of JPEG resources

http://www.yahoo.com/Computers_and_Internet/Software/Data_Formats/JPEG/

Appendix D

HTML Resources

Yahoo directory listing of HTML resources

http://www.yahoo.com/Computers_and_Internet/Software/Data_Formats/HTML/

OneWorld/SingNet WWW & HTML Developer's JumpStation - Page 1

http://oneworld.wa.com/htmldev/devpage/dev-page1.html#doc-a-1-1

Crash course on writing documents for the Web

http://www.pcweek.ziff.com/~eamonn/crash_course.html

A Beginner's Guide to HTML

http://www.ncsa.uiuc.edu/General/Internet/WWW/HTMLPrimer.html

Learning HTML

http://union.ncsa.uiuc.edu:80/HyperNews/get/www/html/learning.html

The HTML language

http://union.ncsa.uiuc.edu:80/HyperNews/get/www/html/lang.html

HTML Tutorial

http://fire.clarkson.edu/doc/html/htut.html

Introduction to HTML

http://scholar.lib.vt.edu/reports/soasis-slides/slides-contents.html

HTML Quick Reference

.http://www.ncsa.uiuc.edu/General/Internet/WWW/HTMLQuickRef.html

Elements of HTML

http://www.w3.org/hypertext/WWW/MarkUp/Tags.html

Writing HTML

http://www.mcli.dist.maricopa.edu/tut/

A basic HTML Style Guide

http://guinan.gsfc.nasa.gov/Web/Style.html

Elements of HTML Style

http://bookweb.cwis.uci.edu:8042/Staff/StyleGuide.html

Style Guide for Online Hypertext

http://www.w3.org/hypertext/WWW/Provider/Style/Overview.html

Composing Good HTML

http://www.willamette.edu/html-composition/strict-html.html

(Netscape's) Extensions to HTML

http://home.netscape.com/assist/net_sites/html_extensions.html

Creating High-Impact Documents

http://www.netscape.com/home/services_docs/impact_docs/

# FEMALE PILOTS IN THE AIR FORCE

Arun Prakash Bala

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored By:
Air Force Office of Scientific Research
Brooks Air Force Base, TX

and

Armstrong Laboratory

July 1995

# FEMALE PILOTS IN THE AIR FORCE

Arun Prakash Bala
Health Careers High School

## Abstract

Pilot gender differences in personality, spatial ability and intelligence will be discussed in this paper. Although research may suggest that males make better pilots, female and male pilots share some of the same characteristics that make successful pilots. Research has demonstrated that variance in spatial ability and intelligence is slight. Female pilots in the United States Air Force today are discriminated against for gender purposes; however, the qualified person, without regard to gender, should be in the cockpit.

# FEMALE PILOTS IN THE AIR FORCE

Arun Prakash Bala

In the Neuropsychiatry Division at Brooks A.F.B. many aviators are referred for testing due to problems seen in their behavior. These behaviors sometimes interfere with the performance of the aviator and that is why the Air Force is interested in solving any deficiencies. The flight surgeons believe that by knowing and understanding their clients they will be able to serve them more efficiently (Reynolds 1). After working with the pilots many doctors begin to see a trend in what pilots have in common. Dr. Raymond King states that the typical aviator is adventurous, courageous, skillful and competent. They usually are masters of complex tasks, are self-confident and typically closer to their fathers. Pilots have no desire for psychological insight and need autonomy. They are largely heterosexual even though female pilots tend to be more like males. Pilots have the need of being in control. This attitude can be attributed to the complete control that they have of the flight when flying. Extremely psychologically and physically healthy, pilots are alloplastic (rather change environment than themselves), self-sufficient, inflexible, direct and intelligent. All of these aspects of their personalities seem to be fulfilled by flying, but there are always exceptions to the rule.

Even though we can make such estimations they always do not hold true. These errors appear because it is impossible to get a perfect representative sample of pilots for any study. The most problems with finding such samples are met when dealing with female aviators. Female aviators have always been discredited because of their sex. Female aviators should not be discriminated against by the United States Air Force as long as they maintain the standards required for becoming a pilot.

Senior Airman Jacqueline D.Bonney is an aerospace neuropsychology technician stationed at Brooks Air Force Base. She has been working in

the Neuropsychiatry division for approximately four years and has had extensive experience testing(psychologically) male pilots. She has noticed several patterns in the pilot's physical behaviors, testing behaviors and their psychological testing posture. The physical behaviors that she commonly observes is that most of the pilots come in with an overconfident attitude that tends to be arrogant. They do not seem intimidated until they see the magnitude of the tests and that the tests are actually quite taxing. When they first look at the contents of the tests they are in a state of awe. This reaction is followed by a puzzled look which is accompanied by certain behaviors. The intimidation that the pilots experience from the psychological testing is demonstrated in such mannerisms as tapping the desk with a pencil, squirming in their chairs, the constant scratching of the head, and hand wringing.

Testing behaviors that Senior Airman Bonney observes are similar to some of the physical behaviors. Typically pilots are very intense and focused when it comes to the matter of completing tasks. Ordinarily when pilots start testing, their facial expressions convey a sense of skepticism which is part of their non-verbal testing behavior. Along with the test taking behaviors there are different psychological factors that are expressed by the testing posture. Those pilots who are not overwhelmed by the test usually sit back in their seats and have a nonchalant attitude about the whole process of testing. Senior Airman Bonney, based on her experience, associates this type of behavior with the pilot's necessity to stay in control. She also notes that when she walks in to check on their progress these pilots are not disturbed by the interruption. But on the other hand, the pilots who appear uncomfortable and perhaps even anxious simply cannot adjust themselves to any interruptions. These are the pilots who perch themselves upon the test and are very guarded about the whole test experience.

Even though Senior Airman Bonney has had only a few encounters with female pilots she can see certain patterns in their characteristics. She said females tended to be a lot like males and had most of the same responses to the testing environment, but women tended to be more agile with the test and finished earlier with proper accuracy. As we can see from Senior Airman Bonney's experiences, the females who were interested in being pilots had the same characteristics of personality that the males had. Also, the females scored about the same on personality scales. Therefore from Senior Airman Bonney's experiences we can understand that both male and female pilots are similar in personality.

Pertaining to personality Alan Feingold states that typically males are more assertive, and tend to be more aggressive and less anxious than females. But this greater male aggressiveness was established mainly on self-report personality scales completed by adolescents and adults. The greater female anxiety was found for measures of general but not social anxiety. Males and females were found to have no difference in self-esteem. Differences in gender in the area of control was concluded to vary by age and only different in the college years. Further differences were seen because females scored higher on the scales of trust and especially tendermindedness. Females also scored higher on extroversion while males scored higher on assertiveness (Feingold 1994).

Many scientists are interested in why people across the world score the same on scales of personality. Some feel that there are biological reasons for personality differences between the sexes. Zuckerman has suggested that gender differences in the traits of dominance and aggression may be caused by biological factors. He said that gonadal hormones probably cause biological sex differences with the traits of dominance and aggression. In his opinion, Zuckerman feels

with some studies that are not reported and those that are falsified, conclusions cannot be made on the higher ability of males in spatial ability.

While scientists question the difference in spatial ability they also are concerned with intelligence. Intelligence is the capacity to acquire and apply knowledge. Contemporary research has indicated that males score higher than females on tests of general knowledge, mechanical reasoning and mental rotations. Females tend to do better on tests of language usage (spelling, grammar) and perceptual speed. In other subjects such as general verbal ability, arithmetic, abstract reasoning, spatial visualization and memory span both sexes did equally well (Feingold 1992).

When we compare the sexes in intelligence an important concept that is forgotten is variability. Variability is the idea that a certain group is not concentrated at one level of performance but is dispersed across the whole spectrum. Feingold states that males are more variable in subjects of quantitative and spatial ability but females are more variable in verbal ability. This idea amounts to a certain sex having a number of intellects but also having a number of underachievers. Certain people argue that the difference in variability is innate but Feingold states otherwise. He says that if this trend of variability was innate then it would be constant across nations (Feingold 1992). Thus, a clear cut difference between the sexes is not conceivable because both sexes are equally intelligent, but it is only their variability that differs and causes the mean of capacity to shift.

In all the above studies a decisive cut could not be made between the sexes. Exceptions to the rule can make some females just as capable on the scales of assertiveness and aggressiveness. Therefore, it would be unwarranted to hold these women down because of their sex. In the area of spatial ability, the absence of a conclusive, concrete shortcoming in females leads us to believe that females can be just as

that the biological factors are more decisive in personality (Feingold 1994).

Conversely, Feingold feels that sociocultural causes are more influential in personality determination even though he agrees that biological factors do have some affect. In his explanation, he states that in prehistoric times the male sex was given greater size and strength which made them superior at hunting, building and waging unsophisticated war. Thus, the man was expected to protect and support the family. The sociocultural aspect comes into play when we still have the attitude of bygone eras therefore causing the males to still be expected to have to act according to the prehistoric male stereotype (Feingold 1994). On the personality scales most males and females score according to their sex, but there are always exceptions to the rule. If a pilot needs a certain personality and a female has it, then her being a pilot is not a problem.

Whether our perception of difference in both genders is biologically or culturally oriented, is a very heated topic among intellectuals. Studies have been performed to decipher the contrasts between the sexes. One concept that is important to the Air Force is spatial ability. Spatial ability is important for pilots to imagine their ambiance in a three dimensional fashion before they actually see the environment. Over the years many studies have been done regarding spatial ability. Some studies state that there is a large difference in this area and that it is large and consistent, but this is not the case (Caplan 1985). Many researchers find that there are no differences in spatial abilities but do not publish their work due to the low personal gain involved. Even the studies that claim that there is a large difference are not reliable because they could be falsified like Porteus' work. Porteus was a researcher who reported that 99 of his 105 studies received higher scores than females on his maze test. Actually, only 4 of the 105 did outscore the females (Caplan 1985). Therefore

on the scales of assertiveness and aggressiveness. Therefore, it would be unwarranted to hold these women down because of their sex. In the area of spatial ability, the absence of a conclusive, concrete shortcoming in females leads us to believe that females can be just as well oriented in spatial relations. Furthermore, intellectually the males and females appear to be the similar only differing in variability. Thus, in seeing no obvious deficiency in female pilots, it would not be appropriate to discriminate against females if they meet all the requirements to be a pilot. In the process of allowing females to be pilots we should not lower the standards because pilots are used in warfare. Crucial wars ended in victory due to the skill of pilots. If some pilots are not as resourceful as others, our national defense will not be as strong. It is a question of common good against individual right. It would be against the common people's welfare if incompetent pilots were allowed to be in the Air Force. Hence, as long as female pilots are capable and fit into the hierarchy, then they should be allowed to serve in the Air Force as pilots.

## References


Bonney, Jacky. Aerospace Neuropsychology Technician, Neuropsychiatry Branch, Armstrong Laboratory, Interview on pilot personality and testing behavior. 26 July 1995, Brooks Air Force Base, Texas.

Caplan, Paula J., MacPherson, Gael M. and Tobin, Patricia. "Do Sex-Related Differences in Spatial Abilities Exist?" American Psychologist July 1985: 786-799.

Feingold, Alan. "Gender Differences in Personality: A Meta-Analysis." Psychological Bulletin 1994: 429-456.

Feingold, Alan. "Sex Differences in Variability in Intellectual Abilities: A New Look at an Old Controversy." Review of Educational Research (Spring 1992): 61-80.

King, Raymond Ph.D. Clinical Psychologist, Neuropsychiatry Branch, Armstrong Laboratory. Interview by author. 14 July 1995, Brooks Air Force Base, Texas.

# A STUDY OF AIR TRAFFIC CONTROLLERS DIETARY QUALITIES

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
Brooks Air Force Base, San Antonio

and

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# A Study of Air Traffic Controllers Dietary Qualities

Heather Castellano
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## Abstract

Concerns have risen in regard to the overall wellness and performance of air traffic controllers on the forward rapid rotation schedule. Since the Professional Air Traffic Controller's Organization strike in the early 1980s, publicity has evoked much concern over the health of ATCs.[2] It can be questioned whether forward rapid rotation schedules compound the already stressful job of air traffic controllers, increasing risks for both the ATCs and air safety. Several aspects of health were studied and evaluated to determine the effects of the rotation schedules on ATCs. Only slight differences was found in the DQI scores between the ATCs and the control group, indicating that the rotation effects are minimal.

# A Study of Air Traffic Controllers Dietary Qualities

Heather Castellano

## Introduction

Forward rapid rotation shiftwork is the basic scheduling pattern used for air traffic controllers at most twenty-four hour United States Air Force air traffic control operations. The shift consists of an eight day cycle. Air Traffic Controllers (ATCs) rotate in this cycle every two days for day, swing and night-shifts and are then given forty-eight hours off. The USAF makes use of the forward rapid rotation shiftwork because it has shown to be most beneficial for most USAF low night-shift workloads. "This schedule has been shown to minimize the chronic desynchronosis associated with slower rotation schedules and theoretically eliminates most of the chronic health problems associated with shiftwork."[1]

As part of a larger study, Capt. Thomas D. Luna and Major Heather Ktenidis sought to concentrate on the dietary habits of air traffic controller's. The original study, Forward Rapid Rotation Shiftwork in USAF Air Traffic Controllers: Sleep, Activity, Fatigue and Mood Analyses, evaluated psychological and physiological effects of the shiftwork. Forward rapid rotation shiftworkers must function at the lowest point of their diurnally oriented circadian rhythms when working the night shift. Their major concern was the effects of the shift rotations on the diurnally oriented circadian rhythms of the air traffic controllers.[1] The purpose of the dietary study was to determine whether there were significant differences in diet between ATCs and a designated control group. Due to the shifting schedule of the ATCs, dietary habits and regular eating times may be disturbed. Consequently, a negative change or decrease in proper nutritional gains may occur affecting the health and job performance of the ATCs.

## Subjects:

Eighteen subjects were involved in the dietary study (the same subjects of the original study). Ten of the participants were male air traffic controllers from both Radar Approach Controls (RAPCON) and control towers.[1] The control group consisted of eight health care technicians; four were female and four were male. Researchers selected this particular group because they were most similar to the ATCs in that they were diurnal workers, with similar ranks, activity levels and lifestyles. The 276 variables of this project dealt with nutritional measurements and participant identifications. There were 6,906 total observations.

**Table 1:** Characteristics of Study Subjects

| Group | Gender | Age Range | Frequency |
|---|---|---|---|
| Air Traffic Controllers | Male | 25+ | 10 |
| Comparison Subjects | Male | 20-24 | 1 |
| | | 25+ | 3 |
| | Female | 20-24 | 2 |
| | | 25+ | 2 |

Methods/Analyses:

To analyze the nutritional data, the data were compiled into SAS. SAS is a statistical program that structures how the data are to be read and analyzed. The advantage of using SAS is that it is an integrated software program which allows absolute control over the administration, analysis and presentation of data.[4] Comparison measures were made between recommended daily allowances of protein and calcium and the actual dietary percentages of each for the ATCs and control group. The National Research Council Recommended Dietary Allowance Table takes into account both gender and age when listing each allowance. Ages twenty-five to fifty are categorized as one unit. Therefore, when computing the RDAs for ATCs, they were assigned age twenty-five, as they were all twenty-five years or older. The ages for the control group varied from twenty-two to thirty-six and were compared accordingly, to the RDA list. After programming SAS to compute the RDAs for each subject, the following variables were entered to be compared among each group: total fat intake, saturated fatty acid intake, cholesterol and sodium intake.

The differences between the two groups were compared using an unpaired t-test. The null hypothesis indicates that the differences in means between groups are zero. The alternative hypothesis is the differences in the means are not zero.[5] When analyzing the results of a t-test, it is important to note that differences in means result in differing or lower p-values (probability, i.e. $p < 0.05$ level) meaning there are significant differences between the groups. In contrast, similar means have higher and more similar p-values which make the null hypothesis more likely to be valid. From this information, it can be concluded that there are not significant differences between the groups. Therefore, each data set is similarly distributed in a normal t-distribution. The latter was the case for the comparison between each group's recommended dietary allowances and the other listed variables. However, protein did appear to be above

average in many cases for the ATCs. When plotting these comparisons using a box plot, the range between ATCs and the control group appeared to be large with dissimilar means, but when the sample size of each is taken into account, it can be concluded that the differences are menial. In a small sample size, one abnormality can cause considerable shift in the mean.

The Diet Quality Index, developed from the Nationwide Food Consumption Survey, was used to compare the dietary standards for each group. The standards for the Diet Quality Index were formed by a multidicsiplinary committee. Into the DQI is incorporated, epidemiological, clinical and laboratory data dealing with dietary information and chronic disease.[3] It provides a list of percentage recommendations for eight dietary factors: total fat intake, saturated fatty acid, cholesterol, vegetables and fruits, starch, protein, sodium and calcium. A score is placed with each factor, 0 ranking closest the recommended percentage of each factor and 2 ranking poorest. Daily sums for these factors are computed giving composite scores. Lower composite scores reflect individual diets closest to dietary recommendations.

Using SAS, the Diet Quality Index Score was computed for each subject. The individual mean DQI was determined over the number of times each subject was observed. Special consideration was given to the measurement of intake for fruits, vegetables and starch. Supplements of vitamin A, K+, C and E were the only attributes measured for each subject (in terms of fruits and vegetables) and were used to compute
the average DQI for the recommendation of fruits and vegetables. Thiamin and riboflavin were used to determine the starch intake of each subject. This gives summary information on the diet qualities for the ATCs and the control group. The DQI scores were then used to compare the overall diet quality between each group. By employing an unpaired t-test, the Diet Quality Indexes were analyzed.

Results:

This research has shown that there are no significant differences in the diets of ATCs and the control group. One problem that may pose further questioning is the fact that the lifestyles of the control group were very similar to that of the ATCs. The diurnal work schedule of the control group could be one reason why there would be no significant differences between the ATCs in dietary habits. Therefore, these groups may have similar means for various other reasons. Although not statistically significant ($p = 0.204$), the ATCs mean was higher at 89.1 percent than the control group at 62.8 for the percent recommended daily allowance of calcium.

The mean DQI for the ATCs was 7.70 and for the comparison was 8.37 producing a p-value of 0.528 (see Table 2). The diet qualities of each group are similar. According to the Diet Quality Index results from the Nationwide Food Consumption Survey, these mean DQI scores fall close to the mean score of 8.6 for their study of American adults (n=5,484).[3] Statistical tests were not used to compare the results of this study to that of the Nationwide Food Consumption Survey. However, with such a small observed difference between this study groups' DQI score and the NFCS DQI study, it could be concluded that the diets of ATCs are not adversely affected by the forward rapid rotation schedule, despite the fact that the control group and ATCs may have some randomization or sampling variability errors.

**Table 2:** Comparison of Mean Values (std)

| Variable | ATC | Comparison | t(16 df) | p-value |
|---|---|---|---|---|
| DQI | 7.70(2.21) | 8.37(2.20) | -0.645 | 0.528 |
| Calories from fat | 35.5( 5.6) | 37.0 (8.10) | -0.466 | 0.647 |
| (%RDA) Calcium | 89.1(48.8) | 62.8(30.92) | 1.323 | 0.204 |
| Sodium | 3418( 816) | 2667(1070) | 1.692 | 0.110 |
| Cholesterol | 260 ( 136) | 189 ( 67) | 1.342 | 0.198 |

## References


1. Luna, Thomas et al. "Forward Rapid Rotation Shiftwork in USAF Air Traffic Controllers: Sleep, Activity, Fatigue and Mood Analyses." BAFB, Texas: Air Force Command Material, 1992.

2. Mason, Kevin T. "Occupational Health and ATC," U.S. Army Aviation Digest. September/October 1989, pp.23-25.

3. Patterson, Ruth E. et al. "Diet Quality Index: Capturing a Multidimensional Behavior," Journal of the American Dietetic Association. January 1994, vol. 1.

4. SAS Procedures Guide: Version 6, Third Edition. Cary: SAS Institute Inc., 1990.

5. Steel, Robert and Torrie, James H. Principles and Procedures of Statistics. New York: McGraw-Hill Book Company, 1960.

# Environmental Quality Technology Synopses and Technology Payoffs

William Brian Cathey

Final Report
Summer Research Program
AL/EQPP

August 11, 1995

**OBJECTIVE:** During my assignment as a High School Apprentice at Armstrong Laboratories, I was involved in an empirical research project describing Environics Directorate technologies in layperson language. The drafts I produced will take the final form of illustrated two- or three-page Technology Payoffs or Environmental Quality Synopses.The final products will be used to tell people all over the world about what the Directorate is producing. My mentor assigned me a total of 14 papers to write and to input into the word processor (Figure 1).

**METHODOLOGY:** My mentor provided me with an example format (Figure 2) and a list of key questions (Figure 3) to be answered in the paper. I researched each project and organized the data into the ormat I was assigned. This research consisted of magizine articles, technical reports, past papers, and video tapes on the project. I also interviewed individual project managers to answer questions not answered in my research.

I began each paper by collecting research and organizing the data into the format I was assigned. I then entered the data into the word processer as well as some finally formating and corrections.

**CONCLUSION:** I throughly enjoyed the eight weeks that I worked at Armstrong Laboratories. I found the experience very educational and rewarding. I worked with great people who were eager to help. I want to sincerely thank my mentors, Mr. Larry Testerman and Mrs. Mary Renolds for their help and patience. The High School Apprenticeship Program is an excelent progam that provides young people an opportunity to work in a professional environment with educated co-workers. This type of program allows young people to see what really goes on after college and gives them a better idea of what field they might want to go into.

(Figure 1)

**ENVIRONMENTAL QUALITY TECHNOLOGY SYNOPSES**

| | |
|---|---|
| SPRAY CASTING | MR JOSH SCOTT |
| IVD ALUMINUM | LT CAROL SMITH |
| RAPID OPTICAL SCREENING TOOL | MR BRUCE NIELSEN |
| BIOREACTOR FOR BIODEGRADATION | MS CATHY VOGEL |
| ANAEROBIC BIODEGRADATION | MS ALISON THOMAS |
| PILOT-SCALE BIOREACTOR | JIM HURLEY |
| SKID-MOUNTED FERROUS SULFATE | LT RAY SMITH |
| BIOFILTRATION | DR. WANDER |
| TRISERVICES TEST SITE | MAJOR MARK SMITH |
| ELECTROLESS NICKEL BATH | LT RAY SMITH |
| IN-WELL AIR STRIPPING/BIOVENTING | LT DAVID KUCH |

**TECH PAYOFFS**

| | |
|---|---|
| BIOREACTOR FOR BIODEGRADATION | KATHY VOGEL |
| PILOT-SCALE BIOREACTOR | MR HURLEY |
| IN-WELL AIR STRIPPING WITH BIOVENTING | LT KUCH |
| SKID-MOUNTED FERROUS SULFATE | LT RAY SMITH |
| BIOFILTERS FOR PAINT SPRAY BOOTHS | DR JOE WANDER |

(Figure 2)

## FORMAT FOR TECH PAYOFFS

**FIRST PAGE**

PICTURE OF TECHNOLOGY

PAYOFF EXPRESSED IN TERMS OF;

1. How much money or time it will save.
3. How it improves an old procedure.
4. How it improves the lot of the worker.
5. How it enhances Air Force Mission.
6. How it improves global quality of life.
7. Other tangible benefits.

**SECOND PAGE**

ACHIEVEMENT

1. What we did?
2. Who helped us?
3. How we did it?

BACKGROUND

1. How was it done before?
2. What were the problems?
3. What had been done to solve problems?
4. What success, if any?
5, Did we build on past efforts? How?

**POINT OF CONTACT**

Name.
Office Symbol.
Telephone Number.

Figure 3)

## ENVIRONMENTAL QUALITY TECHNOLOGY SYNOPSES (EQTECHS)

One-three page illustrated flyer to describe technologies that are either complete or nearly complete.

SYNOPSIS

What it is?
What it does?
What we did?
Who helped us?
How we did it?

RESULTS

What we found out?

APPLICATIONS

Who can use it?
How it can best be used?
Under what circumstances?

BENEFITS

Savings in time.
Savings in money.
Technology Transfer Potential.

DOCUMENTATION: Technical Reports by number, author, title, date published, DTIC Accession Number, if available.

TECHNICAL CONTACT: AL/EQ Project Manager

USER CONTACT: If any.

# *EQTECH*

## Rapid Optical Screening Tool (ROST)

### *SYNOPSIS*

A partnership between DOD, academia, and private industry shows promise of a combined technology that can save millions of dollars in hazardous waste site cleanup costs. The Department of Defense has about 20,000 contaminated sites, many of which require characterization and which may need to be monitored for more than 30 years, costing millions of dollars and comprising about 30- 50 percent of the cost of remediation. The Rapid Optical Screening Tool, a refined and commercialized version of earlier technologies promises to reduce the costs of site characterization, monitoring, and ultimate remediation.

The traditional method of monitoring and characterization has been to use a drilling rig to install monitoring wells within and around a suspected contamination sites. Drilling brings large amounts of soil to the surface the frequently must be disposed of as hazardous waste. This method is extremely slow and expensive. Instead of a drill ROST uses a hydraulic ram to push a slender rod, called a cone penetrometer, into the ground. The ROST is a refined version of the SCAPS (Site Characterization and Analysis Penetrometer System) mated with a Transportable Laser Spectrometer. A personal computer analyzes the light given off by the pollutants and relays the data to the operator.

The laser/fiber-optic spectrometer system, which uses laser-generated ultraviolet light, optical fibers, and spectroscopy, was being developed for hazardous waste site groundwater monitoring. The basic detection approach utilizes fluorescence, much like a glow-in-the-dark toy. Certain substances fluoresce when a specific frequency of light shines upon them. The laser system is unique in that its output may be tuned to the optimum frequency for detecting the pollutants of interest.

Optical fibers are used to transmit ultraviolet light to, and any resulting fluorescence from, the subsurface monitoring points. The spectrometer analyzes the resulting light after it interacts with the soil or groundwater. The system can identify substances such as benzene, toluene, and xylene (BTX) by their fluorescent spectra. jet fuel, which contains BTX, is the most common contaminant at Air Force hazardous waste sites.

Laser spectroscopy technology can provide semiqualitative and quantitative information, on site, in minutes, The system has been tested in the laboratory with detection limits as low as parts-per-billion levels for BTX in water, and can also potentially be used to monitor the progress of site remediation. This provides information quickly at less cost, giving engineers and scientists monitoring site remediation better control of the process. The bottom line is that the entire site remediation process will be more cost-effective.

Armstrong Laboratory's Environics Directorate, along with Unisys Corporation, Dakota Technologies Inc.,and North Dakota State University recieved a Technology reinvestment Project grant for $1.6 million from the Advanced Research Project for further development of ROST. ROST is a transportable laser/fiber-optic spectrometer system which uses laser-generated ultraviolet light, optical fibers and spectroscopy for groundwater monitoring, and hazardous waste site characterization. Certain substances fluoresce when a particular frequency of light shines on them. The laser system may be tuned to the optimum frequency for detecting pollutants of interest. Optical fibers are used to transmit ultraviolet light to subsurface monitoring points and feed back resulting fluorescence into a spectrometer which analyzes

the resulting light. The system can identify substances such as benzene, toluene, and xylene (BTX) by their fluorescent spectra.

The spectrometer was successfully tested in monitoring wells for groundwater and in a cone penetrometer at Tinker AFB, Oklahoma, where it identified and quantified fuel contamination on soils down to about 100 ppm. The technology was also successfully tested in characterizing sites for natural attenuation potential at Plattsburgh AFB, NY; Patrick AFB, FL; Dover AFB, DE; and Bolling AFB, DC.

## *RESULTS*

Studies to demonstrate site amenability towards intrinsic demonstrations were conducted at Plattsbutgh, Patrick, and Dover AFBs. The sites selected for the demonstrations included closed fire figthter training areas, gasoline service stations, and unlined pits used to ignite fuels such as JP-4, waste oils, and other flammable substances. The service stations or hydrant fueling systems have percolated through the vadose zone into the unconfined aquifer. Thepenetrometer determined the aerial extent and volume of oily-phase contamination, and obtained groundwater and soil core samples. To determine site amenability, acquired data were then fed into BIOPLUME(R) II, a computer model for in situ contaminant biodegradation. The cone penetrometer system was able to rapidly locate and define the leading edge of the oily-phase petroleum plume. The technology proved that it can be used to provide timely and accurate data for intrinsic bioremediation modeling.

The Tri-Services conducted a series of laboratory tests and some of the preliminary results are calibration curves with different fuels on various soil matrices. The calibration curve obtained in the laboratory for diesel fuel marine (DFM) on a sand matrix indicates a detection limit that is lower than 30 mg/kg (ppm). The collection of LIF multidimensional data sets (fluorescence emission wavelength, intensity, and lifetime) or WTMs for diesel #2, JP-4, unleaded gasoline, and diesel fuel marine show how each one has a characteristic pattern. These patterns make possible reliable fuel type identification without the need for bringing samples to the surface. In the field the LIF count measurements can be correlated to collected samples and analytical results. To assist in the correlation, several WTMs were conducted at various depths. Color-coded WTMs from the North Tank Area (NTA) and Fuel Purge Area (FPA) at Tinker AFB indicate different fuel types. The shapes of these spectra identify the contaminants as fuel oil at the NTA and JP-4 at the FPA. the fluorescence vs. depth profile from push location 84-L at Plattsburgh AFB indicates narrow bands of contamination are in the "oily phase" which rests just above the water table. Note that discrete sampling at five-foot intervals (25, 30, 35 feet, etc.) could easily skip over the contamination. A series of fluorescence vs. depth profiles taken across a north-south transect at Plattsburgh AFB fire fighter training area show extent of contamination. The contamination traveled directly down from the burn pit and then along the water table.

Currently, these technologies are being further developed and demonstrated within numerous DOD, DOE, and EPA programs. Ongoing research will develop techniques to monitor contaminants such as chlorinated solvents, metal, and explosives which do not naturally fluoresce. Refining this technology is at the heart of site remediation. We can use it to determine if remediation is needed, what remediation technology we should apply, whether the remediation is working, and whether the cleanup effort has been successful, all with the minimum of risk, time, labor, and cost.

## *APPLICATIONS*

The new ROST system will greatly benefit all branches of the military by providing a faster and cheaper way of finding and analyzing underground waste plumes. ROST can be used to not only find and analyze waste site but to repeatedly monitor the sites.

## *BENEFITS*

The ROST has been used successfully at more than 30 fuel-contaminated sites in the United States and Europe. With drilling costs starting at approximately $50/ft, disposal costs rear $450/yd$^3$, and additional cost in chemical analysis this new system is expected to save the Air Force alone more than $100 million at the least. At severly contaminated sites drilling cost can be as high as $2,500/ft, not to mention the dangerous exposure to the workers, but with ROST costs range from $15 to $18/ft, man-hours are reduced, and drilling waste and worker exposure are nearly eliminated.

Armstrong Laboratory and Unisys Corp. signed a Cooperative Research and Development Agreement (CRDA) to commercialize the Air Force-ceveloped laser spectrometer system. The ROST is the proposed product from the commercialization of the laser spectrometer developed for the Air Force by NDSU. It will build upon the previous Envirenics Directorate research by automating the collection and mapping of data, making equipment components smaller and more rugged, and developing a more user-friendly interface to allow use by environmental technicians involved in site characterization and cleanup. ROST also has potential for process monitoring and for medical diagnostices. Initial commercial use will be with cone penetrometers for soil characterization. The DOD will benefit from technologt and knowledge gained; the private sector will receive a highly transferable and profitable technology; the U.S. economy will be helped; and all will benefit from a cleaner environment.

## *DOCUMENTATION*

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# *EQTECH*

## IVD ALUMINUM

## *SYNOPSIS*

In 1988 Armstrong Laboratories began a three phase project, sponsored by the Air Force Civil Engineering Support Agency and contracted to McDonnell-Douglas, to investigate the use of IVD aluminum as a replacement for cadmium electroplating. This technology was developed in the 1970s by McDonnell-Douglas, and uses aluminum, instead of cadmium, to coat aircraft and weapon systems parts. Cadmium electroplating is used to protect components of weapons systems from corrosion, but cadmium is extremely toxic, and is usually electroplated from a cyanide bath, which is also a very hazardous material. The cadmium electroplating process releases about 70 metric tons of cadmium into the atmosphere each year. As more stringent air emission regulations evolve, the wastes of cadmium electroplating will require even more expensive treatment and disposal.

In the IVD process, parts to be coated are cleaned and hung from a rack which is loaded into the top of the plating chamber. The chamber is then evacuated, closed, and filled with argon to a pressure of about 10 micrometers. Then, a high electrical potential is applied between the parts and evaporation sources (called boats) in the chamber bottom, with the parts connected to the negative electrode. this electrical potential ionizes the argon in the chamber. The positive argon ions are accelerated toward the negatively charged parts and begin to bombard them creating a "glow discharge" around them and, over several minutes, remove water vapor and other contaminants from their surfaces leaving the parts clean and ready for coating.

Following the glow discharge, the movable boats in the chamber bottom are powered up and pure aluminum wire is fed onto their white-hot resistively heated elements. The wire evaporates and fill the chamber with aluminum vapor. Some vapor is ionized and accelerated toward the parts, but most is conducted to the parts through collisions with the argon ions. The aluminum condenses on the parts and builds up a columnar porous coating. This process continues for about 30-45 minutes; then the chamber is repressurized and the parts removed. Immediately after coating, they undergo glass-bead penning to densify the coating and are treated with a chromate conversion coating to enhance corrosion protection.

## *RESULTS*

Research on IVD Aluminum was done in three phases. Phase I researchers concluded that 80 percent of the parts being cadmium plated could be switched to IVD Aluminum processing without delay; the other 20 percent fell into three areas of concern to be addressed in Phase II.

Phase II tests showed the IVD Aluminum process could coat inner surfaces as long as there were suitable openings in the parts. When openings were inadequate, inside surfaces could be coated by supplemental processing. When better erosion resistance was needed, a silicon-aluminum alloy could be deposited on parts using the IVD process.

Phase III was the actual demonstration of IVD as a replacement for cadmium electroplating, In July 1991, McDonnell-Douglas representatives began teaching WR-ALC personnel to operate the IVD coater. Soon after, they started developing coating procedures for the aircraft parts which had been plated with cadmium. In the following months, parts were gradually switched from cadmium processing to IVD aluminum coating. By February 1992, so much of the cadmium workload had been changed over that WR-ALC closed its cadmium plating line.

Compared to cadmium, IVD Aluminum gives good corrosion protection, provides adequate adhesion, and increases the service temperature from 450 degrees F to 925 degrees F. In virtually all applications, IVD Aluminum can replace cadmium electroplating of equal thickness. It can also be applied thicker than cadmium.

## *APPLICATIONS*

The IVD Aluminum process can be used to coat nearly all the parts that can be electroplated in less time and at lower cost. As a result of the new IVD Aluminum technology, many Air Logistics Centers have completely shut down their cadmium electroplating lines and now rely on IVD Aluminum instead. This technology is presently being used and all Air Force Air Logistics Centers and several private industries.

## *BENEFITS*

The success of this technology will result in savings of $169,000 per year for cyanide treatment, and $74,753 per year for labor costs at facilities such as OC-ALC and SA-ALC. The process is cost-competitive with cadmium electroplating (Cost pr part - $105 IVD - $105 Bright Cadmium - $142 Low Embrittlement Cadmium - $135 Diffused Nickel Cadmium). Substitution of IVD aluminum for cadmium presents other advantages. IVD aluminum out performs cadmium in preventing corrosion in acidic environments and service tests. Also, aluminum coatings can be used at temperatures up to 950 degrees F, whereas cadmium is limited to 450 degrees F. IVD aluminum coatings can be applied to high-strength steel without fear of hydrogen embrittlement. Aluminum coatings can be used in contact with titanium without causing solid metal embrittlement, and they can also be used in contact with fuels; cadmium is prohibited for these applications. Additionally, IVD aluminum can be used in space applications, whereas, cadmium is limited because of sublimation.

## *DOCUMENTATION*

ESL-TR-88-75
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# *EQTECH*

## SPRAY CASTING

### *SYNOPSIS*

Armstrong Laboratories, along with the help of EG&G and WETO (Western Environmental Technical Office) in Butte, Montana, is developing Spray Casting, an innovative technology that is a practical and cost-effective substitute for electroplating weapon systems and aircraft parts. This project began in 1989 by EG&G, was patented in 1990, and is currently being tested by EG&G in Butte, Montana. It is undergoing three stages of development under a cooperative agreement between AL/EQ and DOE. Spray Casting is a coating process in which parts are sprayed with molten metal rather than dipped in plating baths. This new process of Spray Casting provides a product of equal or better quality than the conventional method of electroplating, but without the harmful toxic bi-products of chromium electroplating.

Spray Casting is a thermal spray process using an inert gas (Argon) propellant. The gas is heated and sent into a supersonic converging-diverging nozzle. This nozzle injects the metal coating into the gas stream in a liquid form where it is broken into tiny droplets, accelerated by the supersonic flow, and sprayed onto the object to be coated. The process uses nonmetal-bearing solutions which cause no toxic waste, and is done in a completely enclosed chamber, unlike the electroplating process.

### *RESULTS*

Two tests were conducted on the Spray Casting process, the first investigated nozzle geometries, mean adhesion strength ranged from 8.3 Mpa (1,202-1,389 psi). Mean microhardness ranged from 12.2-18.9 HV50, and mean grain size ranged from less that 1-8 microns. Results from the first experimental design indicate that orifice location and the three-way interaction between orifice location, orifice diameter, and throat diameter most significantly effect adhesion strength. Mean grain size was most influenced by orifice location and the two-way interaction of orifice location and orifice diameter. Microhardness was most influenced by orifice location and the three-way interaction between orifice location, orifice diameter, and throat diameter. The complex interactions of the variables indicate that all three variables significantly effect the nozzle performance. Therefore, because no one geometry aspect dominated the others, the optimum nozzle geometry was based on the test with the largest microhardness and the smallest mean grain size.

For the second test series, which investigated operating control parameters, mean adhesion strength ranged from 6.20-35.90 Mpa (904-5,220 psi), porosity ranged from 0-34 microns, and mean grain size ranged from less than 1-40 microns. All factors influence adhesion strength and no effect significantly influenced mean grain size. Porosity was most influenced by argon temperature and traverse rate.

Although Spray Casting is still under the final stages of development, experimental studies have shown excellent results. Test results have proven this new process can produce consistently dense and adherent coatings. The process is able to apply thin coatings to parts or produce near-net shape components that accurately reproduce the shape of a mold.

## *APPLICATIONS*

Spray Casting would be used to do the same task as the electroplating process now being used by Air Logistics Centers and other plating centers. This new spray process would replace the chemical baths used in the electroplating of aircraft and weapon systems parts. This new process would produce a product of equal quality while virtually eliminating toxic bi-products.

## *BENEFITS*

Spray Casting is a wonderful new technology that will cut down on cost, production time, and toxic waste. This new process has the potential to reduce production time by as much as 60%, depending on the part being coated. There are also substantial operational and environmental savings with a near 100% reduction in toxic waste. Overspray can easily be collected for reuse or disposal and the waste from this new process in much less hazardous than the present method, therefore greatly reducing disposal costs and environmental danger. In addition, the Spray Casting, unlike electroplating, is done in a completely enclosed chamber and releases no toxic fumes, thus greatly improving operator safety.

Spray Casting would be mostly used by the military but shows great potential use by private industry.

## *TECHNICAL CONTACT*

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## *TECH PAYOFF*

### Pilot-Scale Bioreactor For Biodegradation of Benzene, Trichloroethylene, and other chlorinated solvents

At the end of the Cold War the DoD was left with a projected $140 million pounds of rocket propellant to be disposed of between 1993 and 2005. The Air Force alone can realize a potential savings of $150 million from the recovery and reuse of ammonium perchlorate (AP) from solid rocket propellants. Biodegredation technology, developed by Armstrong Laboratory's Environics Directorate at Tyndall AFB, provides an inexpensive and environmentally acceptable way of disposing of AP in waste streams generated during the recovery process. Biodegradation is effective for wastestreams generated during manufacture, maintenance, and refurbishment of solid rocket motors, and can be used to clean air, water, and soil.

The only method previously available for disposal of AP propellants was open burning or open detonation, both of which cause serious environmental problems. A method has been devised for removing the AP propellant from motor casing by high pressure washout, but the problem of what to do with the AP-contaminated wastestream has been a barrier to recovery, and possible resale and reuse of this propellant.

## *ACHIEVEMENT*

To solve this problem, the Environics Directorate became concerned with development of a method to biodegrade the contaminated wastestreams. A two-stage bioreactor was developed in which an anaerobic bacterium was isolated that would reduce the perchlorate to chloride. After labatory testing, a pilot-scale reactor was field tested at the base industrial area (OT20 site) at Robins AFB, GA. This pilot-scale system was operated for an 8 week evaluation period by Envirogen personnel using groundwater pumped from a contaminated aquifer.

Envirogen Inc., a small business innovative research contractor, teamed with Armstrong Laboratory's Environics Directorate at Tyndall AFB, Fla., have field-tested a two-stage bioreactor designed to break down benzene, trichloroethylene, and several other chlorinated solvents. Contaminated ground water is pumped through the bioreactor where microorganisms "eat" the contaminants. This new reactor is a 2000-liter aerobic bioreactor that reduces perchlorate to chloride. It uses a nutrient handling and extraction section, bioreactor, and a hydrogen sparge. This biodegradation technique, highly effective for perchlorate, may be expanded to biodegrade other components of propellants such as nitroglycerin, resorcinol, and triacetin. The Desirable end-product form the two-stage reactor is carbon dioxide. this promising process was patented by the Air force in April 1994.

Contaminated groundwater was pumped through the reactor for 4 weeks prior to addition of the bacterial inoculum. The FBR effectively removed greater than 97 percent of the 1,2-DCB and greater than 95 percent of the BTEX from the water over the time period including preloading, steady state and spiked phases of operation. During this same time period, aqueous TCE concentration were reduced by an average of 88 percent with a total mass balance demonstrating greater than 84 percent destruction beyond carbon adsorption in the FBR.

Over 210,000 gallons of contaminated groundwater were treated in the pilot FBR during the field trial, with effluent quality close to drinking water standards. Pilot FBR performance was exceptional, with results fully consistent or exceeding the laboratory study. For this reason, the process flow sheet can likely be simplified from a dual-stage to a single-stage biological treatment system.

The GPR was essentially operated as an independent field demonstration for remediating TCE contaminated air. The GPR was capable of effectively treating TCE at concentrations up to 2,000 μg/L air. TCE was reduced by an average of 75 percent in the GPR. This removal rate can be increased to over 90 percent by increasing biomass concentrations in the reactor.

## *BACKGROUND*

The biodegradation technology can prove highly beneficial not only to the government, but also to private industry. This technology is highly transferable and can be used to treat pollutants in air, water, and soil. In the tests conducted all hazardous chemicals were treated to concentrations near or below drinking water standards. This process leaves no hazardous by-products to be dealt with, unlike previous methods of treatment. The biodegradation technology demonstrated in this project is currently available for installation and operation for remediation of contaminated water, either surface of groundwater, and contaminated air originating from air stripping, air sparging, or soil vapor extraction operations.

## *POINT OF CONTACT*

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**Christopher Chadwell report not available at time of publication.**

# THE EFFECT OF HYPERBARIC OXYGENATION AND HYPOBARIC EXPOSURE ON PERIPHERAL BLOOD MONONUCLEAR CELLS

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Sponsored by:
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# The Effect of Hyperbaric Oxygenation and Hypobaric Exposure on Peripheral Blood Mononuclear Cells

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## Abstract

The proliferation of peripheral blood mononuclear cells (PBMC's) under various atmospheric stressors was studied. PBMC's were extracted from remnant blood using the density gradient provided by Histopaque solution. The cells were then exposed to conditions equivalent to 45 feet below sea level and 85,000 feet above sea level. The conclusion made after the experiment was that hyperbaric oxygenation suppresses the proliferation of PBMC's, while exposure to hypobaric conditions increases cell proliferation.

# THE EFFECTS OF HYPERBARIC OXYGENATION AND HYPOBARIC EXPOSURE ON PERIPHERAL BLOOD MONONUCLEAR CELLS

Ai-Hsin Cheng

## Introduction

Peripheral blood mononuclear cells (PBMC's) which includes T-cells, monocytes, and macrophages are the main components of this experiment. These cells are all leukocytes, also known as white blood cells. Some leukocytes are highly phagocytic cells with densely granular cytoplasm and complexly segmented nucleus, like monocytes and macrophages. Other leukocytes, such as, T-cells and B-cells, are also referred to as lymphocytes. These cells have nearly clear cytoplasm, simple or kidney-shaped nuclei, and are involved in the production of antibodies. Each of these cells performs an important role in the immune function of the body. Antibodies produced and regrlated by lymphocytes are necessary in the labeling of foreign particles, bacteria, and viruses for destruction by phagocytes. One function of white blood cells is the release of toxins in an area of damaged tissue in order to kill it and prevent the spread of infection. However, with today's scientific advancements; antibiotics, surgery, and other medical treatments such as hyperbaric oxygenation can kill bacteria and eliminate the need for tissue death. Therefore, decreased numbers of leukocytes in the blood of patients with damaged tissue due to radiation, bacterial infection, or diabetic non-healing wounds would allow the tissue to be saved without endangering surrounding tissues.

Hyperbaric oxygenation (HBO) is a treatment which uses pure oxygen under pressure to maximize tissue oxygen levels. HBO is used in emergency conditions such as decompression sickness and carbon monoxide poisoning. In addition, HBO is also used in non-emergency clinical circumstances including osteomyelitis, radiation tissue necrosis, and compromised wounds. Many hyperbaric oxygenation patients who are treated for damaged or infected soft tissues would benefit from decreased proliferation of leukocytes. Decreased proliferation of leukocytes increase their chances of saving the damaged tissue and decreases the possibility of amputation and tissue removal. Further more, occurrence of fewer lymphocytes allows the blood system to open, allowing better

delivery of antibiotics to infected areas.

A measure of cell proliferation in PBMC populations, when exposed to hyperbaric oxygenation shows the effects of this treatment on the immune system. Cells exposed to the normal conditions of regular air (21% oxygen) at ATM (pressure at sea-level) Provide a negative control. Cells exposed to hypobaric conditions provide a positive control; this is because HBO has been shown to combat the various biological effects of high altitude exposure, as observed during treatment of decompressed sickness.

Problem

The two-part purpose of the experiment was to understand how hyperbaric oxygenation would deviate cell proliferation and to show that rapid decompression would provide a positive control.

Methodology

To extract PBMC's from remnant blood, the whole blood was diluted with phosphate buffered saline solution (PBS). In a 15ml centrifuge tube, the blood was layer on top of a Histopaque solution (ratio of 1 to 1), that provided a density gradient. The tube was then centrifuged at 2,000 rpm for 15 minutes. The resulted tube contained a top layer of blood serum with an underlying layer of PBMC's. PBMC's were carefully removed and washed with 10ml of PBS, centrifuge at 800 rpm for 5 minutes. After decanting the excess solution, media (composing of sterile water, fetal bovine serum, penicillin, and RPMI) was added to the final PBMC pellet. A concentration between 500,000 to 1,000,000 cells per milliliter was acquire. Once the concentration was reached, the cells were distributed into three 12-well micro-titer plates. One plate was left in sterile conditions at 1 atmosphere of pressure. Another plate was exposed to hyperbaric oxygenation at the equivalent depth of 45 feet below sea level (66 psi) for 90 minutes. The remaining plate was placed in an altitude chamber and exposed to the equivalent of 25,000 feet above sea level for 2 minutes. After each plate was exposed to its particular atmospheric conditions, they were incubated for three days. Each well were then counted for cell concentration by the use of a hemacytometer and a microscope on days one and three of incubation.

Results

Three experiments were done using the same procedures at the same cell concentration (500,000). After

three days of incubation, cells exposed to rapid decompression displayed the greatest proliferation; where as cells exposed to hyperbaric oxygenation displayed the least in comparison to control. Cell concentrations of exposed cultures to hyperbaric oxygenation averaged 90,000 cells/ml on the first day and 160,000 cells/ml on the third day.. Concentration of control cell averaged 130,000 cells/ml on the first day and 220,000 cell/ml on the third day. Rapid decompression caused cell concentrations to be at 130,000 cells/ml on the first day and 340,000 cell/ml on the third day. The increased cell proliferation cause by exposure to rapid decompression showed altitude to be the positive control. The short term life -span of PBMC's would explain the low concentrations of cells in comparison to the starting concentration.

Conclusion

Although, this experiment may show that HBO decreases the proliferation of leukocytes in human remnant blood; which, would benefit patients treated by HBO for wounds and tissue trauma; this experiment involved only a few specific types of human peripheral blood cells. This research should be continued, studying leukocytes and their relationships with surrounding cells under similar condition. Further studies of individual components and characteristics of peripheral blood cells under hyperbaric and hypobaric stressors should be pursue. A method to take into consideration would be to repeat the experiment using feeder cells. Feeder cells increases the life span of neutrophils by providing antioxidant protectants. This would enable better measurement of the long term effect under different atmospheric conditions.

Effect of Laboratory Experience

Lacking the proper laboratory experience I was hesitant at first. However, after the experience I had while conducting this research, I am now more confident of my laboratory skills. This experience increased my interest in scientific research and prepared me for the real world of science.

References

Knowledge obtain during the time spent while conducting this experiment are to be given credit to the various scientists, physicians, physiologists, and personnel of the AL/AOH, special recognition to Dr. Edward Piepmeir, Dr. Larry Krock, and Major George Kemper .

# RADIATION DETECTION AND MEASUREMENT

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FINAL REPORT FOR:

HIGH SCHOOL APPRENTICE PROGRAM

ARMSTRONG LABORATORY

JULY 28, 1995

# RADIATION DETECTION AND MEASUREMENT

The operation of any radiation detector basically depends on the manner in which the radiation to be detected interacts with the material of the detector itself. An understanding of the response of a specific type of detector must therefore be based on a familiarity with the fundamental mechanisms by which radiations interact and lose their energy in matter.

The majority of the samples dealt with in my laboratory involve the detection and measurement of alpha particles, beta particles, and gamma rays. The alpha and beta particles are detected on The Gamma Products G5000, a gas flow proportional counting system, and the gamma rays are detected on a sodium iodide detector.

The Gamma Products G5000 detector uses a heavily-shielded proportional detector for the detection and analysis of radiation. The operation of this detector is based on the way that different types of radiation events interact with the detectors. Alpha events will generate a large signal in the sample detector but will not pass through to the guard detector. Beta events will generate a small signal in the sample detector and will not pass through to the guard detector. Gamma events will generate a small signal in the sample detector and will almost always pass through into the guard detector generating a signal there at the same time.

When a charged particle enters a proportional detector, it collides with gas molecules causing gas ionization to occur. This ionization creates positive ions and free electrons that travel in opposite directions. A charge is converted to a voltage pulse by a pre-amplifier, amplified and shaped by the main amplifier and then analyzed

by the MCA as to its amplitude. This data is then displayed on the screen and may be printed out at the time of the operators command.

Depending on the operation voltage applied to the detector, it will respond differently to the alpha, beta, and gamma radiation events. If the voltage is too low, there is no charge collection and the detector will have no response to any event regardless of its type or the energy deposited in the detector. At an increased voltage, the detector will respond to the alpha but not to the beta or gamma events. This method is referred to as voltage discrimination.

All proportional counters will register some counts even with no sample present under the detector therefore background counts must be run daily. These counts are the result of cosmic radiation and electronic noise. Since the processes that generate these background counts are random processes, the process of measuring the background is going to fluxuate. This means that the errors associated with background subtraction add to the uncertainty of your measurement. In some circumstances they may be a major part of your total uncertainty.

Cosmic radiation can greatly affect the outcome of results. The four inches of lead shielding in the G5000 System removes the less energetic components of cosmic radiation.

The sodium iodide detector depends on scintillation and the release of this energy as pulses of light, known as fluorescence. The sodium iodide detector absorbs energy from the ionizing particle. The scintillator then emits energy in the form of fluorescent light. This light is then converted to one or more electrons. These electrons collect on the anode and are deposited on a capacitor, where they produce a voltage signal.

At the beginning of each week an environmental background check must be performed. If any type of contamination is suspected background may need to be run every day. Background count time is 1200 seconds.

Quality assurance is performed on Monday, Wednesday, and Friday of every week. Quality assurance is run for 600 seconds. The reported peak channels must be compared with the established peak channels of Co 60 and Cs 137.

To screen swipes for gross gamma radiation, 10 swipes must be placed on the detector face. After initiating a count and allowing it the proper amount of time to run, a report will be generated. This report will indicate a gross gamma result for the ten swipes. If all values on the final analysis report are less than 50 pCi, it is acceptable to report all ten swipes as being less than 50 pCi. If a report of greater than 50 pCi is generated for any group of ten swipes, isolate the "hot" swipe by process of elimination. Once the "hot" swipe/swipes are found, they must be run on the gamma spectroscopy system for qualitative and quantitive determinations.

# Programming Psycho-Motor Tests Involving Risk Assessment

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# PROGRAMMING PSYCHO-MOTOR TESTS INVOLVING RISK ASSESSMENT

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## ABSTRACT

In order to determine how well risk assessment tests measure individuals' behavior, I, under the auspices of Dr. Joshua Ben Hurwitz, programmed a psycho-motor test to measure behavior. The test involved two arcs rotating around a gun in the middle of the computer screen. The gun fired bullets at two different speeds. Subjects had one shot per trial, and gained a number of points inversely proportional to the time and lost a constant number of points (always more than the number gained).

The program was written in object Pascal, specifically Borland Pascal 7.0. With extensive comments and the graphical instructions, the main program was 759 lines. However, the nature of the task necessitated the creation of two other programs. One created the data file which initialized the bullet speed, arc size, arc speed, and arc starting position; it was approximately 123 lines. The other file, approximately 375 lines, took the data file output from the main program and rearranged it and formatted it for importation into a statistical analysis program for Windows™ called SPSS™.

# PROGRAMMING PSYCHO-MOTOR TESTS INVOLVING RISK ASSESSMENT

## WILL P-Z. CLARK

### *Introduction and Discussion of Problem*

One of the questions about risky decision making is whether individual differences in risk taking are due to differences in risk perception or risk acceptance (Elander, West & French, 1993; Snapper & Edwards, 1973). Some individuals may take risks because of inadequate processing of information that bears on the risk. Others may process the information correctly, but choose to take the risk anyway.

On psychomotor tasks such as driving or piloting, this distinction is important because psychomotor abilities may relate to risk perception and to the ability to avoid a negative outcome in a risky situation. Given that individuals with poor psychomotor abilities are poor at estimating real-time parameters such as distances, times and velocities, they would more likely develop faulty perception of the risks involved in real-time tasks. According to this hypothesis, poor real-time processors would be more likely than good processors to take inordinate psychomotor risks, as when a beginning skier with poor psychomotor abilities decides to ski down the advanced slope after having practiced only twice on the beginner's slope.

Another hypothesis, however, is that previous experiences with high-risk psychomotor tasks influence the tendency to take risks. According to this assumption, inaccurate real-time processors avoid taking psychomotor risks because they have learned that they tend to make more errors than other individuals. Accurate processors, on the other hand, have learned that they can take risks and still avoid making errors.

This assumption addresses the fact that being trained to perform many types of complex psychomotor tasks, such as driving, flying, and skiing, involves taking risks. That is, no individual who does not take any risks could acquire these skills because there are risks inherent in the training process. If real-time psychomotor abilities are developed and maintained as a consequence of having engaged in activities such as these, then individuals who have well-developed psychomotor abilities would be more likely to take risks than those with lower ability levels. With its emphasis on the relationship between current behavior and previous successes and failures, this hypothesis is reminiscent of the behaviorist approach toward modeling performance (Millenson & Leslie, 1979).

A third hypothesis is that psychomotor abilities and risk-taking tendencies are independent of each other. According to this conception, each individual has a target level of risk, which represents the amount of loss they are willing to tolerate. Individuals make choices about whether to engage in a risky psychomotor activity so as to maintain this target level. If risk levels decrease below this target level, then the individual's behavior becomes riskier. If risk levels go too far above the target, then behavior becomes safer (e.g. Aschenbrenner & Biehl, 1993; Wilde, Claxton-Oldfield & Platenius, 1985). However, according to this hypothesis, individuals' target levels bear no relationship to their psychomotor abilities.

These three hypotheses were tested by analyzing data from a task in which subjects tracked, aimed and fired at one of two targets. One of the targets was more difficult to hit than the other, requiring greater psychomotor precision for tracking and aiming. Also, this target was riskier than the other because hitting it produced greater rewards while missing it produced greater losses. Finally, the targets differed in that subjects had been trained more on one than on the other. Thus, subjects could choose targets on the basis of how much experience they had with them, as well as on how difficult and risky it was to hit them.

In addition to being presented with choices varying in difficulty and risk level, subjects were also under pressure to give a timely response. Time pressure was implemented by decreasing the potential gain as a subject's response time increased. After 6 seconds, subjects could not gain any points for hitting either target.

Time pressure was employed because evidence from the literature suggests that subjects filter stimulus information when given a limited amount of time to make a choice (e.g. Ben Zur & Breznitz, 1981). In the filtration process, only the most salient attributes of the stimulus are employed when generating a response. If a tendency to make risky choices has been automated as a consequence of previous experiences, then risk-related attributes (i.e. those associated with the payoffs) should be more salient than other attributes (i.e. those associated with difficulty level) for those subjects who tend to take risks. Thus, for high-risk subjects, risk-related attributes should automatically be processed under time pressure, while the other attributes are processed to a lesser extent.

Aside time pressure, payoffs and target difficulty, the study also employed a grouping variable derived from an external measure if risk-taking tendencies. Subjects were divided into high- and low-risk groups according to their responses on a risky-activities questionnaire. The questionnaire asked them to indicate how frequently they participate in a number of activities, half of which were considered risky. As a validity check, subjects were also asked to judge the risk levels associated with each activity.

Using the results of this questionnaire, we were able to assess the relationship between the subjects' risk levels and their acceptance of risk in choosing targets. As discussed above, risk levels could be independent of psychomotor ability. In this case, high-risk subjects should always select the more difficult target more often than

low-risk subjects, regardless of past performance. However, if a subject's riskiness is driven purely by differences in real-time abilities (i.e. risk perception), then high-risk subjects should select targets that are more difficult than the ones they can perform well on given their past performance.

If risk levels are based purely on past successes and failures on risky psychomotor tasks, then high-risk subjects should select targets that are optimal for their performance level, whereas low-risk subjects should always select the easiest target available. Note that, according to this hypothesis, high-risk subjects might always choose the more difficult target, even if their initial performance is poor on these targets. However, their superior psychomotor abilities would lead to an improvement in performance on the harder targets. Therefore, even though it would be initially risky to try those targets, the efforts of high-risk subjects should succeed in the end.

Thus, the main focus of the study will be to assess, both for high- and low-risk subjects, the relationship between target selections on the one hand, and prior and subsequent performance on the other. According to a pure rational model, subjects should make selections that maximize their performance levels. However, given a relationship between psychomotor abilities on the one hand, and faulty risk perception or acceptance of inordinate risks on the other, then the results of the current study should show that the choices subjects make on risky psychomotor tasks do not maximize performance.

## Methodology

We needed to develop a program that presented two moving targets to the subject, and allowed the subject to track and aim at one of the targets, and then fire at it. The program we developed passed through several revisions. The original plan for the program called for the targets to be two filled circles. They moved from the top of the screen to the bottom, with each having a different size and speed. The gun, which fired small, circular bullets, was centered in the x-direction on the screen and located at the bottom. The program would award points if the subject hit a target and would subtract points if the subject missed both targets. Points were inversely related to size of the circle that was targeted, and were directly related to the speed of that circle.

However, after careful consideration, we ruled out that idea because the faster circle would come closer to the gun and thus afford the subject the opportunity to gain an inflated score. We then had the idea of having the gun placed in the center of the screen, with the circles rotating around it. This revision lasted for quite a while and elicited most of the algorithms which would be used in the final version.





At the particular laboratory to which I was assigned, I found many other programmers with experience in creating this type of test. With the help of fellow employees, I learned some object-oriented programming in order to use the object libraries which would handle the complex code necessary to build smooth animations, draw the various objects, accurately record response times, etc. The huge repository of pre-existing code sped up the development process and allowed me to concentrate on more high-level code, rather than reinventing the wheel.

More than anything else, using the animation libraries helped create a smooth animation where all speeds and timing issues are independent of machine speed. This created a framework that shields the user from the details of page-flipping (storing two screens worth of data in the video card and switching between them very quickly) and of synchronizing with the vertical retrace (making sure page flipping and drawings only execute when the video card updates the monitor). Also, dealing with the mouse, the joystick, the foot pedals, and the keyboard became simply a matter of calling the right function to control the required input device.

In addition, the libraries also provided higher level functions. To some extent, the problem became one of finding these predefined functions and using them to the fullest extent. For example, I used a previously created function called MoveDir (which, given a starting position, angle, and distance, returns an x and y coordinate) to position the bullet and draw the gun. I also had to learn about the nature of sprites (moving portions of the screen which can pass over a background image and not clobber it), which I found in libraries. Although the object-oriented nature of libraries hides many of the details of using sprites, I still had to learn the protocol used in the object and how to manipulate the sprites in the page-flipping context of the animation object.

However, in some cases, I had to deal with lower-level ideas. In order to erase the text that accompanied each circle in its circular orbit around the gun, I had to use an array, indexed by the current video page, to erase the text on the non-visible page (the active page). It took me nearly a day to get that idea down and implement it.

In order to keep the frame rate (the number of frames per second) as high as possible, I tried to optimize in as many ways as possible. I tried clearing the entire screen instead of erasing each component individually, both of which took about the same amount of time. However, I discovered later that I needed to erase components separately in order to complete the instructions as planned.

At this stage, after the project seemed near completion, we reviewed it and decided that shooting at circles might not be the best choice because the bullet can hit different parts of the circle at different times. We then decided to use arcs circling around the gun as the targets. That way, a bullet fired at an arc will reach any part of it at the same time. Before the final method emerged, I tested various methods for speed, including rotating sprites and rotating figures (objects in one of libraries) before concluding that using one of Borland Pascal's built-in functions (arc) would work best in this situation. It takes the radius of the circle, starting point (in degrees), and the ending point (also in degrees) and draws an arc of circle. Using this method, an arc of a desired speed can be created simply by giving it a speed[1] (in radians or degrees per second) and multiplying by a timer (another convenient bit of code provided by the animation libraries).

At this major revision point in the history of project, I also changed the algorithm to locate the text from one explicitly dealing with long cosines and sines to one using a previously created object (MoveDir ,the same one used to draw the gun and the bullet). Thus I could easily position both the text and the arcs by giving each an angle and a radius.

In addition to the major graphical changes introduced at this point in the project, we finalized the plan for creating the data. According to this plan, the first sixty trials would be practice, and in those the subject would be presented with two arcs of equal size, speed, and point value (in all trials one arc starts at a random position and

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[1] All the measurements in the program use radians to simply statistical calculations in the eventual analysis of the data collected by this program.

the other starts 180 degrees away). In the practice trials, each arc would travel at 2/15 Pi radians per second, is 2/15 Pi radians wide, and is worth 1,250 points. As time passes, the points gained (on the bottom of each point block in fig. 1) would decrease, whereas the points lost would remain constant (on the top of each point block in fig. 1). In this part, the subject would theoretically learn his/her proficiency at hitting this particular arc, heretofore known as a "standard" arc.

Then, in the real trials mode of the program, the size and speed of one arc would vary (not within a trial) while the other was always a standard arc. Arc-2 could be any one of six different combinations of size (1/15 Pi radians, 2/15 Pi radians, 3/15 Pi radians) and speed (1/15 Pi radians per second, 2/15 Pi radians per second, 3/15 Pi radians per second). However, it could never be 2/15 Pi radians wide and travel at 2/15 Pi radians per second because those are the specifications for the standard arc (always arc-1).

The program that creates the data file generated five of each particular combination of size and speed per thirty trials. It then shuffled the trials so the subject got what appeared to be a random mix of arcs. This allowed for easy analysis as the numbers of each type of arc were always the same, regardless of the arrangement. Finally, for every ten trials during practice trials and every thirty trials during real trials, the time it took the bullet to reach either arc changed from 2/3 second to 2 seconds (the program warned the subject before a switch).

Each trial generated one line of text in a data file; it recorded the current trial number, the reaction time (time from the beginning of the trial until the subject fires the gun), the gun's angle at the time the subject fired the gun, the time it took for the bullet to reach either arc, the width of each arc, the starting position of each arc, the speed of each arc, the ending position of each arc, whether the subject missed or hit and, if hit, the arc hit, and the number of points lost or gained.

The final version of the program formatted the data file for a statistical analysis program called SPSS™. It took all the data and assigned each trial a unique variable name with seven characters, indicative of exact type of trial the data represents. For example, one variable might be TSRF1NL, where the

T = average of all of this type of arc's reaction time;
S = the subject hit the standard arc in this trial;
R = this trial was part of the real trials;
F = the gun fired a fast bullet in this trial;
1 = block 1 out of four in group of bullet speeds;
NL = the bullet was normal speed but was larger than normal.

## ***Results and Conclusion***

My tour ended just as I finished the program to format the data for SPSS™. Thus, we did not have time to formally analyze any data. Simply observing the test subjects revealed one rather striking outcome: males performed consistently better than females. Several factors could have contributed to these results:

1. Males generally take more risks than females and the test could have favored riskier behavior
2. Males have better psycho-motor skills
3. Males have prior experience with joystick, specifically playing arcade-style video games

I learned a great deal in the area of object oriented programming, which will certainly help my future life with computers. Also, most of my prior programming experience dealt with data structures, traditional computer science course material. This job has helped me to learn more about graphics and graphics algorithms on IBM™ compatible computers.

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**Associate did not participate in program.**

# SELECTION OF GENES FOR THE REDUCTION OF PERCHLORATE

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Final Report for:
High School Apprentice Program
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# SELECTION OF GENES FOR THE REDUCTION OF PERCHLORATE

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## ABSTRACT

The purpose of the research conducted during my apprenticeship was to clone genes for perchlorate degradation from *Wolinella succinogenes* strain HAP-1. This strain has been previously determined to reduce perchlorate. There is a need for a biological degradation of the perchlorate because it is a major component of large rocket motors which are being disposed of by the Air Force. Currently there is a system of reduction facilitating the HAP-1 reduction capabilities at Tyndall Air Force Base, but this system is inefficient due to the nature of the host strain of the reductase, HAP-1. In my research I selected for the reductase gene from the HAP-1 and transfected it, by the use of cosmid vectors and lambda phage, into the alternative host of *E. coli*. In doing successfully doing this, the Air Force will be able to create a more efficient system for the degradation of perchlorate using the transfected *E. coli* which now carry the perchlorate reductase genes.

# SELECTION OF GENES FOR THE REDUCTION OF PERCHLORATE

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## HYPOTHESIS

To establish a gene library from strain HAP-1 DNA and screen the library for the expression of perchlorate reductase. From this the genes responsible for the reduction of perchlorate can be transferred into a microbial host with minimal growth requirements, such as *E. coli*.

## BACKGROUND

Ammonium perchlorate is used extensively as an oxidizer in the defense industry. Common applications of this perchlorate salt are in propellants, explosives and pyrotechnic composites. (Urbanski, 1988.) The main use or ammonium perchlorate is as an oxidizer in solid rocket propellants; often times composing up to 70% by weight. Large amounts of aqueous ammonium perchlorate is produced from the manufacture, refurbishment, and maintenance of these large motor rockets. While the ammonium perchlorate is relatively stable in these solutions, this solution must be treated to remove the ammonium perchlorate before it can be released into the wastestream.

Recoiling technologies for ammonium perchlorate have been developed and tested. While some of the systems claim to be "closed loop systems", they are only capable of reclaiming 80% of the ammonium perchlorate in the feeder solution and the dissolved ammonium perchlorate from the water recycling will continue to accumulate and compromises the product quality. In addition, the waste stream from this type system would require further treatment before disposal. Also, manufacturers of ammonium perchlorate have outfalls which are unable to be recycled.

Previously, materials containing ammonium perchlorate were disposed of in a practice known as open burning/open detonation in which the materials were ignited in an open pit. Similar methods of disposal also employed was the static firing of the large motor rockets. In both of these cases the products of combustion, such as hydrogen chloride, chlorine, ammonia, and nitrogen, entered the atmosphere untreated and uncontrolled. In some instances , the defense industry has taken some of their more concentrated solutions of ammonium perchlorate and sold them to commercial explosive manufacturers. Eventually, disposal of solid rocket engines will generate large quantities of dilute waste streams containing ammonium perchlorate which require treatment.

Recent trends in the United States militaryhas made alternative methods for the disposal of ammonium perchlorate most necessary. The Secretary of Defense announced that the Air Force plans to retire the Minuteman II fleet. This deactivation of 492 missiles will generate over 34 million tons of propellant. The elimination of the ammonium perchlorate in all these rockets by static firing or open burning/open detonation techniques would release 3,000 tons of HCl into the atmosphere (Smith, 1990).

Intense public opposition to the static firing and open burning/open detonation techniques indicate that this will not be a viable form of disposal. The Environmental Protection Agency (EPA) has required that all open burning/open detonation sites be classified as hazardous waste disposal sites. As a result the cost of the open burning/open detonation. Eventually, all open burning/open detonation will be prohibited by the Clean Air Act.

For the reasons previously stated, there has been a push to find alternative methods for the disposal or reuse of the ammonium perchlorate found in propellants. One of the options being pursued is the bioremediation of the ammonium perchlorate found in the propellants.

Biodegradation of the ammonium perchlorate is viewed as the method that potentially requires the lowest capital investment and offer lower maintenance costs than other methods of disposal. This method of biological treatment has the least amount of public opposition. Consequently, the United States Air Force has determined that the biodegredation of ammonium perchlorate is the most environmentally and economically sound method of treatment.

In 1963, Hackenthal et al. described several species of bacteria capable of reducing perchlorate ion to chloride ion. (Hackenthal et al., 1963). While their study focused on the mechanism which govern the reduction of both nitrate and perchlorate, the study defined that there were several bacteria capable of perchlorate at constant rates. Perchlorate was degraded in this study both anaerobically and aerobically. It was proposed that industrial waste waters containing perchlorate could be treated biologically, by Korenkov in 1974 (Korenkov, 1974). In this study raw communal sewage was utilized as a nutrient source. Researchers on this project identified a minimum amount of biochemically required oxygen (BRO) introduced with the communal waste for optimum degradatioonVibrio dechloraticans Cuznesove (B-1168), the organism identified by the patent that resulted in this research was able to with stand only 300 mg/l perchlorate.

Experiments conducted by Aerojet General in, reported in 1989, resulted in a system using a mixed culture capable of sustaining the degradation rate of 59 mg/l in an anaerobic fluidized bed reactor (Manville RedmedTech, 1989). Failures in this system proved to be problematic to sustain the system.

Further study was conducted by Attaway and Smith on bacteria they sampled from the Denver Municipal sewage treatment plant's anaerobic digester. ( Attaway, 1993). The sample studied in the lab was capable of anaerobically degrading perchlorate at a high considerably higher than all others previously studied. Since Attaway and

Smith had obtained a culture capable of degrading perchlorate, the isolation and characterization of the isolate responsible for perchlorate degradation had been achieved.

Addition studies at Tyndall Air Force Base, conducted by Wallace et al. identified a strain of bacteria identified as HAP-1 which was capable of degrading perchlorate. The strain was isolated from a municipal anaerobic digestor for its ability to reduce greater than 7,000 ppm perchlorate in wastewaters. HAP-1 is capable of the reduction of both perchlorate and chlorate for energy and growth. Antibiotic resistance profiles, utilization of carbon substrates and electron acceptors, demonstrated physiological characteristics similar to that of *Wolinella succinogenes.* Comparison of 16S rRNA sequences showed only a 0.75% difference between HAP-1 and *Wolinella succinogenes.* The only notable differences between HAP-1 and *W. succinogenes* is HAP-1's ability to reduce perchlorate and chlorate and it's inability to reduce nitrate, a characteristic of *W. succinogenes.* Due to the exceptionally high rate of reduction by HAP-1 of ammonium perchlorate it has been incorporated into a treatment process for the removal of perchlorate from rocket motor wash water. A pilot plant was engineered including both an anaerobic and aerobic phase of reduction. The system was designed to include to be mechanically reliable allowing long periods of operation without significant upsets dues to human and mechanical error. While the actual reduction of perchlorate occurs in an anaerobic reactor, an aerobic reactor was necessary in order to reduce the biological and chemical oxygen demands as well as the ammonium in the anaerobic superfluent. Once this system was in place and running on Tyndall Air Force Base, it became evident that the system was proved to have many limitations. Strain HAP-1 has many nutrient requirements which are met by the addition of large amounts of Brewer's yeast. Because of the superfluent of the anaerobic reactor, an additional aerobic reactor was necessary. The manpower necessary to operate that system included agitation of the brewer's yeast for four hours allowing for a 12 hour period allowed for the settling of sludge as well as the removal of supernatant and the disposal of sludge waste.

Upon the commencement of my studied at the Armstrong Laboratory, it was suggested by William Wallace, PhD., that I research the possibility of transferring the genes responsible for the biodegradation of perchlorate from the strain HAP-1 into a microbial host with minimal growth requirements such as *E.coli.* In addition, I was to establish a gene library from strain HAP-1 and screen that library for the expression of the perchlorate reductase.

## METHODOLOGY

### ISOLATION OF GENOMIC DNA FROM STRAIN HAP-1

The strain HAP-1 bacteria from a saturated culture were lysed and the proteins removed by digestion with proteinase K according to a published procedure (Current Protocols in Molecular Biology, 1991). In addition, the cell wall debris and the remaining proteins were removed by selective precipitation with CTAB, and the high molecular weight DNA is recovered from the resulting supernatant by isopropanol precipitation. Following this basic procedure, an additional purification is performed on a cesium chloride gradient. This procedure was followed in order to obtain the large amounts of exceptionally clean genomic DNA required for the construction of the genomic library.

### QUANTITATION OF DNA BY ABSORBANCE

UV spectrophotometers measure the ability of solutes to absorb light at specific wavelengths. The light absorbed at a given wave length can yield information concentration information. By examining the concentrations of the DNA isolated in the previous step, the purity o the solution can also be analyzed. By viewing the concentrations at 260 and 280 nm on the spectrophotometer, the DNA isolated was determined to be both clean and concentrated enough to proceed.

### PARTIAL RESTRICTION OF HAP-1

Restriction, in molecular biology, describes a bacterium's natural defense to recognize DNA entering it as foreign, in indicated by its inability to be properly methylated, resulting in the degrading of the invading bacterium. This natural process has been put to use by molecular biologist who now use these isolated restriction enzymes to cut molecules reproducibly and predictably. In each case, the restriction enzyme recog nizes a specific sequence, although that sequence differs from different species and strains. Many types of restriction enzymes have staggered cuts and leave behind 'sticky' ends which are of great use in rejoining fragments. With this knowledge, I was able to cut the strain HAP-1 DNA with the restriction enzyme Sal-1 and BamH-1. Only a partial restriction of the strain HAP-1 DNA was performed in order to increase the possibility of obtaining the reductase of perchlorate intact in later steps of my research. The choice to use BamH-1 andSal-1 was based on its previous performance in the lab and the its known site on the cosmid vector to be used later in the experimentation. While many experiments were performed using various concentrations of Sal-1 and BamH-1 and varying length of reaction, the decision to proceed with a one hour restriction of strain HAP-1 and a two hour restriction by BamH-1 was made.

COMPLETE RESTRICTION OF pPZ101 VECTOR

While only a partial restriction of the HAP-1 DNA by both Sal-1 and BamH-1 was performed, a complete restriction of the cosmid vector pPZ101 was the next step in my research. Cosmid vectors are often used in this type of research due to their small size, their replication mode of plasmids, and the ability to be packaged in-vitro into bacteriophage lamvda heads. The cosmid vector pPZ101 was chosen for its previous performance in the lab and its ampicillin resistance, which would be later used as a selection marker. A complete digestion of the pPZ101 vector DNA by both the Sal-1 and BamH-1 was desired in order to increase the number of vector DNA fragments available for ligation with the restricted strain HAP-1 DNA. The restriction reaction of pPZ101 DNA by both Sal-1 and BamH-1 enzymes ran for two hours.

LIGATION OF RESTRICTED HAP-1 DNA AND pPZ101 VECTOR DNA

Ligation is the process by which a DNA ligase joins DNA strands together at cohesive compatible ends which are resultant of restriction with the same restriction enzyme. This process forms a recombinant DNA molecule. The sticky ends which result from the digestion by the restriction enzyme can easily form hydrogen bonds with complementary unpaired sequences. DNA ligase is then added to form strong covalent bonds. The genomic HAP-1 DNA was added with the vector DNA in a six to one ratio. This ratio is necessary in order to insure that the vector and HAP-1 ligated with each other and not fragments of their respective kind, which would form cancatamers. This reaction took place in a water bath which is kept at 16 C for 12 hours.

LAMBDA PACKAGING

Bacteriophage lambda is a double-stranded DNA virus with a genome size of approximately 50 kb. In the bacteriophage lambda particles, the DNA is in the form of a linear molecule with single-stranded complementary ends. After entering the host bacterium, the DNA circularizes through the pairing of cohesive ends and is transcribed as a circular molecule during the early phase of infection. The bacteriophage lambda is capable of serving as a vector due to its nonessential region of approximately 40 kb located between the right an d left arms of the lambda DNA. Into this stuffer region the ligated DNA can be packaged for eventual transfection. This phase of the experimentation was carried out under the direction of the instructions provided in the DNA packaging kit from Boehringer Mannheim Biochemical Company.

TRANSFECTION OF *E.coli*

Transfection is the process by which the bacteriophage lambda transfers pieces of DNA into cells and these genes are expressed in the new host. They infect the bacteria cells by attaching to the cell surface and injecting the genes they are carrying at receptor sites. The recombinant HAP-1/pPZ101 DNA packaged in the lambda transfected two strains of *E. coli,*

HB101 and DH5alpha. This procedure was also carried out in accordance with the directions from Boehringer Mannheim, with the exception of the two restrictions enzymes used and the to strains of *E.coli* to be transfected. The most efficient transfection was that of the Sal-1/HB101 transfection, with approximately 2,225 colonies grown on Luria and ampicillin agar plates.

CREATION OF GENE LIBRARY

In order to preserve the transfected *E. coli* fro further experimentation in the future, it was necessary for me to store make a gene library from the transfected *E. coli* and to store this library cyrogenically in glycerol stocks. Fifteen thousand colonies were selected and cataloged to be stored in the glycerol stocks. This made up the HAP-1 gene library from which the perchlorate reductase was to be selected. The library was constructed from 10% glycerol with Luria broth.

EXPRESSION OF PERCHLORATE REDUCTASE FROM GENE LIBRARY

From the gene library established in the previous step, I selected 450 colonies from which I would test for ampicillin resistance as well as the reduction of perchlorate. The resistance towards ampicillin serves as an indicator of the vector insertion into the recombinant *E.coli*, the vector was encoded for this ampicillin resistance. Th reduction of perchlorate was indicated by the presence of Rezasurin in the agar which would turn pink when oxidized, indicating that the perchlorate in the agar had been reduced. One hundred and nine of the four hundred and fifty colonies turned pink in the presence of perchlorate and rezasurin. These one hundred and nine colonies were further tested for perchlorate reduction on plates containing ammonium perchlorate in the agar as well as plates that did not contain ammonium perchlorate, this insuring that the recombinant *E. coli* was reducing the ammonium perchlorate and not another substance in the agar. None of the 109 colonies plated without ammonium perchlorate turned pink in the presence of ammonium perchlorate. Ten of these colonies were selected for plasmid analysis.

PLASMID ANALYSIS

A miniprep of the plasmid DNA according to published procedure was performed. This alkaline lysis method is an easy way to obtain isolated plasmid DNA. By examining this isolated DNA on an agarose gel, I was able to see that I had succeeded in transfecting the *E. coli* with the recombinant HAP-1/ pPZ101 vector ligation, as indicated on the gel by the distinct bands identified by the lambda standard.

CONCLUSIONS

From my research involving the HAP-1 DNA I am able to conclude that it is possible to isolate the genes responsible the biodegradation of ammonium perchlorate from the *Wolinella succinogenes* strain HAP-1 and to Transfect them, by the use of lambda packaging into an alternative host. In addition, a gene library of the strain HAP-1 was established which will be of great help to any others who continue research on this strain.

APPLICATIONS

The alternative host to the perchlorate reductase developed in my research has a great number of applications in the defense industries search for an economically and environmentally sound way of disposing of ammonium perchlorate wastestream which results from the deactivation of large rockets. This strain of *E. coli* developed can be used to replace the present HAP-1 biodegradation system in use by the Air Force at this time. Since *E. coli* requires only a lactose nutrient supplement, versus the brewer's yeast requirement of the HAP-1, the cost of operation of the bioreactor will be greatly reduced. The current cost of Lactose is 28-30 cents/il while the cost of Brewer's yeast is 37-40cents/lb. In addition, it may be possible to eliminate the second aerobic reactor from the system since the supernatant from the anaerobic reactor will not be so great. The use

of this strain may prove to be the most economical and environmentally sound form of treating ammonium perchlorate presented.

LIMITATIONS

While it appears that my research on the reduction of perchlorate by HAP-1 and the transfection of the reductase genes into an alternative host seem successful, I acknowledge that the research I conducted left many things unanswered and much research still to be completed. Due to the short length of time I was able to spend in the laboratory, I was unable to fully quantitate the results of my research. In order to most assuredly state that I engineered an alternative host in a strain of *E. coli* in which the perchlorate reductase is expressed, further quantitative analysis, to determine the rate and amount of perchlorate reduced by this recombinant strain is necessary. Also, further research on the genes which encode for the reductase is needed to fully understand the biochemistry of the reaction. Subcloning of large cloned fragments into the smallest essential gene fragment which encodes for the perchlorate reduction reaction is needed. Addition studies to examine the rates of perchlorate reduction would help to quantitate my research. A comparison study between the efficiency of the perchlorate reduction in *E. coli host* with lactose versus HAP-1 with Brewer's yeast. Eventually, the DNA sequence for genes encoding perchlorate reduction could be determined. While I did establish a gene library and successfully transfect *E.coli* with the reductase, this is only the beginning of research on the biochemical pathway of reduction of perchlorate by the stain HAP-1 reductase.

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**Associate did not participate in program.**

# ELECTROMAGNETIC FIELDS AND CELLULAR ADHESION

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# ELECTROMAGNETIC FIELDS AND CELLULAR ADHESION

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## Abstract

Cell membrane exteriors are known to have a net negative electric charge, thus electrostatic forces are important to cell adhesion. By calculation, it was determined that the electrostatic repulsive force between cells should be extremely large. Comparison of these forces to experimental values determined by others showed that electrostatic screening and probably additional physical mechanisms must be reducing the amount of repulsive force between two cells. Electromagnetic fields can alter the charge on the membrane exterior, and thus the electrostatic force; therefore, they may affect cellular adhesion. This could be important to the field of cancer therapy and in health and safety considerations, since altered adhesiveness is a characteristic of cancer cells. Therefore an experiment to test the effect of electromagnetic fields on cell adhesion was proposed.

# ELECTROMAGNETIC FIELDS AND CELL ADHESION

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## Introduction

Modern society is using increased amounts of electromagnetic energy in the conduct of its affairs. Ambient levels of electromagnetic energy at several frequencies, from 60 Hertz, through the megaHertz regions and even into the gigaHertz domain are increasing when one looks across time as measured in decades. Since so much of the life process is mediated by electrical charge and electric fields, it is no wonder that there has been substantive research activity addressing the biomedical effects of electromagnetic fields. This report examines in a preliminary way the influence of electromagnetic fields on cell adhesion.

## Discussion

The cell adhesion process is complex. Simplistically, a few ligands from one cell attach to receptors from another cell. If these few bonds can keep the cells in contact long enough for more ligand-receptor bonds to form, the cells will remain attached (Singer, 1992). Cell adhesion molecules (CAMs) assist in the process and act as molecular glue to bind the cells. Elisabeth Bock (1991) reports five groups of CAMs: the integrin family, the cadherin family, the selectin family, the immunoglobulin family, and the epidermal growth factor family. The real puzzle though, is not how the cells bind, but how they come into contact with one another.

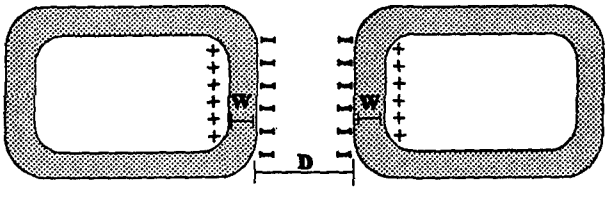


**Figure 1**

**Orientation of charges over two cells in close proximity. Positive charges gather along the inside of the membrane, while negative charges line the membrane. D represents the distance between cells. W represents the width of the cell membrane.**

The electrostatic force between particles of like charge causes repulsion, whereas the electrostatic force between oppositely charged particles causes attraction (Parsegian, 1973). Cells are known to carry a net negative electrical charge on their external surface; thus the electrostatic force between two cells should cause them to repel (Bell, 1978). However, some cells exhibit the completely opposite phenomenon.

The electrostatic force of repulsion, $F_e$, between two cells can be calculated using Coulomb's Law, which states that

$$F_e = \frac{kq_1q_2}{d^2} \quad ,$$

where k is the electric constant, $q_1$ and $q_2$ are the charges of the particles, and d is the distance between particles. In the case of two cells, the charges are identical, and the electric constant k is approximately equal to $9.0 \times 10^9$ Newtons square meters per square Coulomb.

Figure 1 shows the orientation of the charges of two cells. To a first approximation, four sets of electrostatic forces occur when two cells draw near one another. The most obvious force is the repulsion between the two negative membrane exteriors. Indeed, there is also a repulsion force between the positively charged cell interiors, and there is attractive force between the negative membrane exterior of one cell and the positive interior of the other cell and vice-versa. Therefore, using Figure 1,

$$F_e = \frac{kq^2}{D^2} + \frac{kq^2}{D+2W^2} - \frac{2kq^2}{D+W^2} \quad (1).$$

The average cell membrane width is 100Å, and the interstitial distance may vary. A hydration layer prevents the membranes from any closer approach than 10-15Å (Zhelev and Needham, 1994). Zhelev and Needham (1994) reveal that structures on the surface of the membrane such as bound polymers and groups of charged particles can act to separate the lipid parts of the membranes to distances of hundreds of Ångstroms. Using intercellular values such as these, calculation of the force shows that the repulsion should be so great that cells should fly apart at extremely high rates, in fact, faster than the speed of light. This calculation will be exhibited later. Since cells do not fly apart at the speed of light, indeed they most often do not fly apart at all, some other physical mechanism must be taking place.

Experimentally determined adhesion strengths also make it apparent that there are factors inhibiting the electrostatic repulsion. Adhesion strength is defined as the minimum force needed to separate bound cells. Tha and Goldsmith (1988) report that the force required to separate red blood cell doublets is approximately 1.43 nano-Newtons (nN). In addition, Bell (1978) suggests that the force per bond is 0.04 nN. These tiny forces are not indicative of the great force of repulsion which should be occurring between cells.

So what is it that reduces the forces of repulsion between two electrically negative cells? It has been found that electrostatic screening eliminates a great deal of the large repulsion force between cells. It results mainly from "changes in the concentration of charged species in the vicinity of charges and dipoles" (Lockhart and Kim, 1993). The net negative potential of the exterior of the cells attracts cations, which counter the charge, and repels anions (Parsegian, 1973). As additional positive ions are attracted, negative ions are brought along. Ions from the surrounding medium cluster around the charged cells, and Nordenström (1994) explains that the attracted ions form two types of layers around the particle: a firm Stern layer, and a movable Helmholtz layer. Some replacement of monovalent $Na^+$ with divalent cations such as $Ca^{2+}$, $Mg^{2+}$, or $Ba^{2+}$ also causes a reduction of the repulsive force (Chien, 1978).

Because electrostatic interactions play such a large part in cell aggregation and adhesion, it is only logical to hypothesize that electromagnetic fields may affect cellular adhesion. Jehle (1969) states that "all that is needed is a change in the ionic condition of the medium, which implies a lack of neutralization; similar or equal types of cells are then apt to repel each other." Electromagnetic fields can alter the charge on the cell membrane, and thus they may alter cellular adhesion (Rubinstein, 1990).

Studies of altered adhesiveness are of biological importance since tumor cells differ from normal cells in properties associated with adhesion. Electromagnetic fields are currently being used to treat cancer (Yu-Lin and Deruo, 1994), and experiments are also being performed (Omote et al., 1990; Nordenström, 1994; Salvatore et al., 1994) which combine electromagnetic fields and chemotherapy. Data from these experiments have shown that EMF alone causes some tumor regression, although not statistically significant amounts; however when both EMF and chemotherapy are used, the effect is enhanced. It is not yet understood exactly why this happens.

For this reason, the authors propose an experiment to test the effect of electromagnetic fields on the strength of cellular adhesion. This could be accomplished using micropipet aspiration. As in Tha and Goldsmith (1988), coupled cells could be secured on one side by a holding pipet, with an aspirating pipet on the other which would apply pressure. Using this technique, it would be possible to measure the force needed to pull the cells apart while an electromagnetic field is directed perpendicular to the pipets and between the adherent membranes. Forces should also be measured in the same manner in an EMF-free environment.

In the following, the authors will provide a more detailed discussion of repulsion force and associated calculations, adhesion strength measurement, electrostatic screening, EMF therapy, and the proposed experiment.

Methodology - repulsive force and associated calculations

Repulsive forces were calculated using equation 1, which states that

$$F_e = \frac{kq^2}{D^2} + \frac{kq^2}{D+2W^2} - \frac{2kq^2}{D+W^2} \quad .$$

Factoring $kq^2$ out of the equation, gives

$$F_e = kq^2 \left(\frac{1}{D^2} + \frac{1}{D+2W^2} - \frac{2}{D+W^2}\right) \quad .$$

Then letting D = nW where n is any positive real number,

$$F_e = kq^2 \left(\frac{1}{n^2W^2} + \frac{1}{(2+n)^2W^2} - \frac{2}{(1+n)^2W^2}\right) .$$

Now it is possible to factor $W^{-2}$ out of the equation, giving

$$F_e = \frac{kq^2}{W^2} \left(\frac{1}{n^2} + \frac{1}{(n+2)^2} - \frac{2}{(n+1)^2}\right) \quad (2).$$

Once this equation is achieved, the electrostatic repulsive force can be determined as either a function of W, the membrane width, or as a function of n, the ratio of the distance between cells to membrane width. Remember that k is approximately $9.0 \times 10^9$ $Nm^2 \cdot C^{-2}$, and the charge q on the exterior of a single side of a cubic cell may be calculated using the following relationship:

$$C = q / V ,$$

where C is the capacitance of the part of the cell membrane in contact with the other cell, and V is the membrane voltage. The capacitance per unit area of the cell membrane is $5.7 \times 10^{-6}$ Farads per square centimeter. Since the surface area of one face of the assumed cubic cell of side 0.001 cm is $10^{-6}$ $cm^2$, the capacitance of the adherent surface of a cell membrane is $5.7 \times 10^{-12}$ Farads. The typical membrane voltage of a cell is 0.06 volts. Using these values, the charge on the membrane exterior of one side of a cubic cell equals $3.42 \times 10^{-13}$ Coulombs.

Using an average membrane width of 100Å, the charge on the one side of the membrane of the cubic cell, and the electric constant, electrostatic repulsive force can be calculated as a function of n, the ratio of intercellular distance to membrane width. When the cells are 50 Å apart, n = 0.5, and the force of repulsion between two cells is approximately 34.4 Newtons. This is a very large force between such tiny particles. For additional repulsive force values, see Table 1.

**Table 1: Repulsive Forces**

| D (Å) | n | F (N) |
|---|---|---|
| 25 | 0.25 | 157.0 |
| 50 | 0.5 | 34.4 |
| 75 | 0.75 | 13.2 |
| 100 | 1.0 | 6.4 |

From the repulsion force, the accleration and velocity of a cell can be determined.

Assuming a cubic cell of side 0.001 cm, the volume of the cell can be figured as $(0.001cm)^3$, or $10^{-9}$ $cm^3$ . The density of a cell is approximately 0.001 kilogram per cubic

centimeter. Given the density equation,

$$\rho = \frac{m}{V} \quad ,$$

the mass of a cell equals density times volume, or $10^{-9}$ cm$^3$ x 0.001 kg/cm$^3$. So the mass of the cell is $10^{-12}$ kg.

If the electrostatic force of repulsion is equal to the net force acting on the cell, using the cell's mass, the acceleration of the cell can be determined using Newton's Second Law,

$$F_{net} = ma \quad .$$

Acceleration equals the repulsive force divided by the cell's mass, $10^{-12}$ kg. So, at the 50 Å separation distance, where n = 0.5 and force ≈ 34.4 N, the cell's acceleration should be approximately 3.44 x $10^{13}$ m/s$^2$. This appears to be a phenomenal acceleration of a tiny cell for, if the cell were in free space, within 8.72 microseconds the cell would be at the speed of light (approximately 3.0 x $10^8$ meters per second). Additional accelerations can be found in Table 2.

**Table 2: Acceleration**

| F (N) | a (m/s$^2$) |
|---|---|
| 157.0 | 1.57 x $10^{14}$ |
| 34.4 | 3.44 x $10^{13}$ |
| 13.2 | 1.32 x $10^{13}$ |
| 6.4 | 6.4 x $10^{12}$ |

However, the cell is not in free space or vacuum but is immersed in a liquid that offers viscous friction and retards motion. Thus, a more realistic assessment of the cell's velocity can be calculated directly from the force, using Stoke's law concerning friction on an immersed spherical particle (here we are assuming that as regards friction the spherical particle does not depart substantially from the friction on a cubic cell). Stoke's law involves the drag on a spherical particle as it moves through a viscous fluid. If the fluid were ideal, with zero viscosity, the streamlines around the paricle as it moved through the fluid would be perfectly symmetrical, and thus the pressure at any point upstream would equal the pressure at the corresponding point downstream. The resultant force on the sphere would be zero. However, when the fluid is viscous, there is a drag on the particle (Sears et al., 1983). Stoke's law states that the viscous force $F_r$ is

$$F_r = 6\pi r\eta v \quad ,$$

where $\eta$ is the viscosity of the fluid, r is the radius of the sphere, and $\upsilon$ is the steady-state or "terminal" velocity of

the particle. This is explained as follows. The force balance equation for a particle in a liquid is

$$F_{net} = F_a - F_r \quad ,$$

where $F_{net}$ is the net force acting on the particle, $F_a$ is the force applied to the particle, and $F_r$ is the viscous force of resistance acting against the particle. In the case of a cell in its surrounding fluid medium, we assume the applied force on the cell is the electrostatic force of repulsion, $F_e$. Therefore,

$$ma = m\frac{dv}{dt} = F_e - 6\pi r\eta v \quad .$$

In the steady state, that is when dv/dt = 0, the viscous force must exactly balance the electrostatic force acting on the particle. Thus,

$$F_e = 6\pi r\eta v \quad .$$

The viscous drag is experienced by particles of all shapes, but only for a sphere is it able to be calculated using the simple analytical expression above. Therefore, we assumed that inscribed in the cubic cell of side 0.001 cm is a sphere of radius 0.0005 cm. An approximate viscosity of 0.01 dynes seconds per square cm, or $10^{-7}$ Newtons seconds per square cm, was acquired from Sears et al. (1983) for water at room temperature. Let $x = 6\pi r \eta$. Then, terminal velocity can be determined by dividing the repulsive force by the value x. At n = 0.5, when the force of repulsion is 34.4 N, the velocity of the cell should be approximately $3.65 \times 10^{10}$cm/s or $3.65 \times 10^{8}$ m/s, still faster than the speed of light. Of course, the terminal velocity is the greatest speed the object can obtain in the case under consideration here, but nonetheless, the speed achieved seems unrealistic and, clearly, some other mechanism to reduce the repulsive effects, beyond viscosity, would seem to be needed. Table 3 presents additional terminal velocities.

**Table 3: Terminal Velocity**

| $F_e$ (N) | v (m/s) |
|---|---|
| 157.0 | $1.67 \times 10^{9}$ |
| 34.4 | $3.65 \times 10^{8}$ |
| 13.2 | $1.40 \times 10^{8}$ |
| 6.4 | $6.79 \times 10^{7}$ |

A QuickBasic computer program which calculates the force of repulsion, the acceleration of a cell, and the velocity of a cell can be found in the appendix.

Adhesion strength is generally measured using micropipet aspiration, the pulling apart of coupled cells using suction. This procedure works using doublets, or by assembling two cells.

Doublets can be assembled by holding a single cell with a pipet and then maneuvering a second cell close to the other and gently apposing them. If the two cells remain in contact when the aspirating

pipet releases the second cell, they have been successfully joined.

Pre-existing and assembled doublets can then be pulled apart by micropipet aspiration to determine the force needed to break the bonds. In Tha and Goldsmith (1988), the doublet is secured to the holding pipet using mouth suction. Then the aspirating pipet is manuevered to contact the doublet from the other side, and a negative pressure is applied until the cells come apart. See Figure 2 for a diagram of the apparatus, and see the following for a short discussion of the proposed experiment.

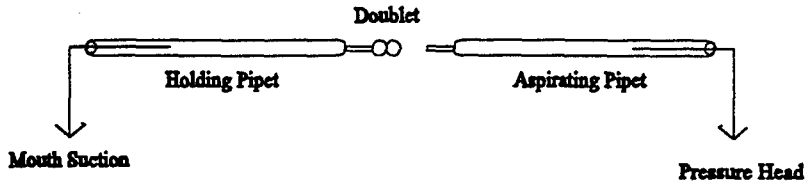


**Figure 2**

**A holding pipet secures the doublet by mouth suction, and the aspirating pipet pulls the doublet apart with the use of pressure.**

**(Adapted from Tha and Goldsmith, 1988.)**

Electrostatic screening is the process by which positive ions in the medium surrounding the cells cluster around the negatively charged cells, thus greatly reducing the force of cellular repulsion. According to the authors' calculations outlined above, without electrostatic screening, at a separation distance of 50 Å, two cells of membrane width 100 Å should experience a force of repulsion of 34.4 N, and should fly apart at $3.65 \times 10^8$ m/s.

This does not occur. Therefore we turn to ionic screening as a possible mechanism whereby a large part of the repulsive force may be eliminated.

Following Chien (Blank, 1980) the electrostatic repulsive force $\sigma_e$ in dynes per centimeter squared can be calculated as

$$\sigma_e = 0.064\ IRT \tanh^2\left(za\frac{\zeta}{4kT}\right) e^{-\kappa d} \quad ,$$

where I is the ionic strength in moles per liter, R is the gas constant, k is the Boltzmann constant, T is the absolute temperature, z is the cationic valency, a is the electronic charge, $\varsigma$ is the zeta potential , d is the intercellular distance and $\kappa$ is the Debye-Huckel function given below:

$$\kappa = (8\pi a^2 N I z^2/1000\epsilon kT)^{1/2} \quad ,$$

where N is Avogadro's number and $\epsilon$ is the dielectric constant of the medium. Working in dextran solutions Chien estimates a maximal repulsive force of approximately $10^7$ dynes per centimeter squared (at zero intercellular distance). For the cellular interface considered above, this corresponds to a separating force of 0.1 milli-Newtons, well below the figure of 34.4 Newtons computed at 50 Å neglecting screening. This separating force of 0.1 milli-Newtons is profitably compared to single adhesive bond strengths of 0.04 nano-Newtons (Bell). Division of 0.1 milli-Newtons by 0.04 nano-

Newtons implies that there must be 2.5 million bonds holding two cells together at each face. This computed number is much larger than the estimate of 45 bonds per adherent cell face determined for red blood cells by Tha and Goldsmith (1988); however, it is known that the Debye-Huckel approximation has significant error in non-dilute solutions so that screening may be even more profound than indicated above. On the other hand, it is possible that still other physical forces are important in cell adhesion. Specifically, can surface tension effects, separate from molecular bonding, play a role (Ohki and Ohshima, 1985), and how important are van der Waal's forces in the process of cell contact and adhesion (Nir and Andersen, 1977)?

Several experimental cancer therapies that involve electric current, and some which couple electromagnetic fields and chemotherapy, have evolved in recent years. These methods have been tested on animal and on human carcinomas, both *in vivo* and *in vitro*. Experimental treatment on humans is limited; for, it is only performed as a final effort to save the cancer patient.

Omote et al. (1990) tested the effects of a combination of pulsed magnetic field (PMF) and mitomycin C, an antitumor drug, on two rat tumors both *in vitro* and *in vivo*. The experimenters studied four groups for each tumor type: the first received no treatment, the second was given only chemotherapy, the third was exposed only to a pulsed magnetic field (200 Hz/40 Gauss), and the fourth was exposed to a magnetic field one hour after receiving the antitumor drug. Rats which lived longer than three months were considered survivors.

All of the untreated rats died in a mean of 21.3 days. In addition, eight of twelve rats in the group given only mitomycin C, and seven of thirteen rats exposed only to PMF died within three months. However, of the rats given mitomycin C with exposure to PMF, only three of thirteen died within three months. The results of Omote et al. show that pulsed magnetic field and mitomycin C in combination is effective in slowing tumor growth and increasing life span.

Salvatore et al. (1993) cultured MCF-7 breast carcinoma cells and prepared wells containing 25,000 cells per well. Then the anti-neoplastic agents methotrexate, cyclophoshanide, and doxorubin were added to the wells in groups of increasing concentrations. The wells were subsequently incubated either with or without magnetic field exposure (15 Hz/1 Gauss).

Using the endpoint of neoplastic cell viabiltiy as their criterion, the experimenters found that the electromagnetic field exposure of MCF-7 combined with the antimetabolite methotrexate enhances the anti-neoplastic effect of the drug.

Björn Nordenström (1994) was the first to test electrostatic therapy in humans. Each of his three voluntary patients had heard of the use of EST in animal experiments. These patients were suffering from cancer which traditional methods could not slow or destroy.

The first patient had lung cancer which was growing ino the wall of the chest and into the mediastinum. A rod-shaped electrode was inserted into the mediastinum. Over six days, the electrode was charged from -83 volts (V) to -200V and then to +230V.

The patient experienced discomforts such as increased perspiration and pressure inside the right lung. Unfortunately, the tumor did not change size, and, six months after treatment, the patient died.

Along the same lines, an electrode was inserted into the mediastinum of the second patient, who was to receive

two electrostatic therapy sessions. This patient suffered from pulmonary and mediastinal metastases. For the first treatment, the voltage across the electrode was alternated from +10V to -10V each day for five days. One month later, the second treatment was administered. The patient received 24V with alternating polarity every day for one week.

The patient experienced only increased perspiration. After treatment, the tumors in his lungs had decreased in size, but the mediastinal metastases remained.

The third patient had developed pulmonary metastases. A coiled electrode was inserted into the mediastinum. Varying voltages were applied to the electrode for 6.5 days.

When the voltages reached over +300V, the patient's head ached, and he felt pressure in his chest. After EST, the small tumors had decreased in size, and one had also become less dense. Because the tumors remained, chemotherapy was administered. Six months later, no metastases could be seen.

Nordenström found that electrostatic therapy alone results in regression of tumors in 2 of 3 cases, and that added effects are observed when chemotherapy is used in conjunction with EST.

Using electrostatic therapy, Yu-Ling and Deruo (1994) treated fifteen human patients by inserting an electric pole into the patient's thigh and connecting the other pole to a copper plate placed underneath the patient's bed. The patients were treated continuously for two weeks with either low voltage, high voltage, or high voltage and a high magnetic field. Each day the positive and negative poles were switched. The experimenters varied low voltage from 200 to 1200 volts and varied high voltage from 500 to 2500 volts. The magnetic field which accompanied high voltage was 12,000 Gauss.

Of fifteen patients, results were as follows: 2 showed tumor regression, 5 displayed no change, and 9 exhibited advance of tumors. Five of the fifteen subjects felt a relief of symptoms, while six experienced side effects from the electricity. At higher voltages, more pronounced side effects such as dryness of mouth, sweat, headache, dizziness, nausea, insomnia, and depression were observed by some of the patients.

Although Yu-Ling and Deruo could not show a strong correlation between the use of electrostatic therapy and the reduction of tumors, they are further exploring its effects.

Results - A Proposed Experiment

The authors propose an experiment to test the effects of cellular adhesion. Using an apparatus similar to the one used by Tha and Goldsmith (1988), we can perform micropipet aspiration. As in Figure 3, a continuous water system coupled to a micrometer-driven water manometer could be used to secure the doublet to the holding pipet. Tha and Goldsmith (1988) used mouth suction for this purpose; however, this seems unstable. In addition, we would not want to risk possible exposure to the electromagnetic field of the person performing this task. Next, the aspirating pipet could be connected to a continuous water system and a digital pressure transducer which would measure the negative pressure used to pull the cells apart. Finally, and electromagnetic field generator could be positioned so that the field would be directed perpendicular to the pipets and between the adherent cell membranes.

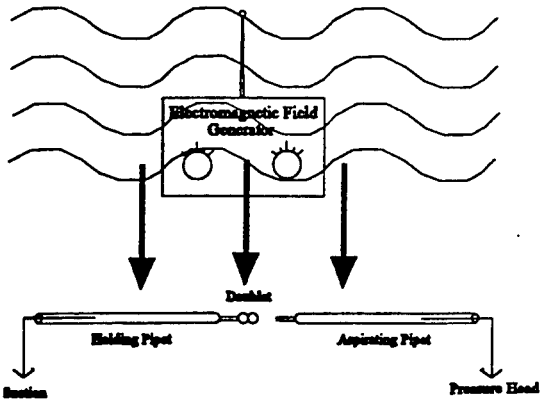


**Figure 3**

**Experimental Apparatus: A holding pipet secures the doublet by a continuous water system coupled to a micrometer-driven manometer. The aspirating pipet pulls the doublet apart with the use of negative pressure. An electromagnetic field is generated perpendicular to the pipets so that it runs between the adherent cell membranes.**

## Conclusion

The authors have derived an equation from which the unscreened electrostatic force of repulsion between two cells can be calculated to a first approximation. We have provided evidence that physical mechanisms such as electrostatic screening, and probably others, must be acting to reduce the repulsive force between cells. In addition, we have provided a discussion of the use of electromagnetic fields in cancer therapy, and we have outlined an experiment to test the effects of EMF on cellular adhesion.

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Appendix

The following QuickBasic computer code will calculate the force repulsion between two cells and a cell's consequent acceleration and velocity. If the computer is equipped with a graphics package, the program will also display semilog plots of the data versus n, the ratio of separation distance to membrane width.

```
INPUT "Membrane width in Angstroms=", MWIDTH
REM*****MEMBR is membrane width in meters
MEMBR = MWIDTH * 1e-10
REM*****k is electric constant in Newtons square meters per square Coulomb
k = 9.0e+09
REM*****MEMBV is membrane voltage in volts
MEMBV = 0.06
REM*****MEMBC is contact surface membrane capacitance in Farads
MEMBC = 5.7e-12
REM*****MEMBQ is membrane charge in Coulombs
MEMBQ = MEMBV * MEMBC
pi = 4 * ATN(1)
REM*****flvisc is fluid viscosity in Newtons seconds per square centimeter
flvisc = 1e-7
REM*****radius is cell radius in centimeters
radius = 0.0005
REM*****mass is cell mass in kilograms
mass = 1e-12

DIM force(3000)
DIM mult(3000)
DIM accel(3000)
DIM vel(3000)

factor = 0
delfactor = 0.001

FOR I = 1 TO 3000
factor = I * delfactor
```

```
numer = 2*(3*factor^2 + 6*factor + 2)
denom = (factor*(factor + 1)*(factor + 2))^2
mult = numer/denom
force(I) = k * MEMBQ^2 * mult(I) / MEMBR^2
divisor = 6 * pi * flvisc * radius
REM*****vel is velocity in centimeters per second
vel(I) = force(I) / divisor
REM*****natural log of velocity
vel(I) = LOG(vel(I))
REM*****accel is acceleration in meters per second squared (neglects friction)
accel(I) = force(I) / mass
REM*****natural log of acceleration
accel(I) = LOG(accel(I))
REM*****natural log of force
force(I) = LOG(force(I))

NEXT I
        OPEN "accel" FOR OUTPUT AS #1
                FOR I = 1 TO 3000
        PRINT #1, I * delfactor, accel(I)
                NEXT I
        CLOSE #1

OPEN "force" FOR OUTPUT AS #1
                FOR I = 1 TO 3000
        PRINT #1, I * delfactor, force(I)
                NEXT I
        CLOSE #1

OPEN "vel" FOR OUTPUT AS #1
                FOR I = 1 TO 3000
        PRINT #1, I * delfactor, vel(I)
                NEXT I
        CLOSE #1

END
```

# "RADIATION DOSIMETRY" WHAT IS IT ?

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Final Report for:
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August 1995

# "RADIATION DOSIMETRY" WHAT IS IT ?

Nicholas Flores
St. Gerard Catholic High School

Abstract

The procedure of issuing and receiving personal radiation dosimeters for both monthly and quarterly periods was studied. More than twice the number of dosimeter badges were used for a quarterly period than for a monthly. The office in which I worked was very cramped for the number of people employed. However everyone did an excellent job processing the badges. Ergonomics was a concern also, but when I left they were addressing the problem and I am confident that they will make that office a better, more comfortable place to work.

# "RADIATION DOSIMETRY"
# WHAT IS IT ?

Nicholas Flores

## Introduction

Since 1947 the United States Air Force has been monitoring its personnel for radiation exposure. This process began at Wright-Patterson AFB and was moved to Brooks AFB in 1981. It consists of: issuing badges to personnel in the field, receiving them, reading the badges for any exposure, keeping records of everyone on the program, annealing the badges, and reissuing them to personnel in the field. This process is done for two different kinds of monitoring periods, monthly and quarterly (which is every three months).

## Methodology

I thought the best way to study this process would be to actually perform the day to day tasks of the technicians. So, from 12 June to 4 August 1995, I worked in the Radiation Dosimetry Branch at Brooks AFB. I did everything but read the badges themselves. I did not do this because I did not have the security clearances to do so. I did, however, put incoming badges into read files, help to keep files on personnel in the field, and issue annealed badges for both the monthly and quarterly periods. I also did some office work that needed to be done, such as answering phones, calling customers with questions, and looking up information on the computer networks.

## Results

After spending time in the Radiation Dosimetry Branch of the Air Force, I've come to realize the monitoring of personnel in the field is very important. There are many different radioactive sources used in the field and even more people who work with them. The newer Panasonic dosimeters are a big step forward from the older Harshaw badges which were a major improvement over the old film badges. The newest dosimeters will be arriving soon; and with bar code scanning, most of what is done in this office now will be cut out. Who knows what the future holds for dosimetry ? One thing is for sure, it will be here for a long while to come.

References

I would like to extend a special thanks to all the military personnel who helped me with my research and for teaching me about dosimetry. Thank you Capt. Bonano, Lt. Afinidad, SRA Magana, and especially thank you TSgt. Wildfeuer. I would also like to thank all the civilian personnel for all there help and training in the procedures used for the dosimetry program. Thanks to Mr. Bump, Laurice Cockrum, Rosie Ramirez, Annette Valdez, Mary Lou Rosas, Lynn Simmons, Tina Grujic, Juan Serna, and Sandra Ortiz. This was a very interesting and educational experience for me and I hope to return next summer.

# KNOWLEDGE SURVEY AND ASSESSMENT

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Knowledge Survey and Assessment

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Abstract

The task of this summer's tour was to assist in the development of the Knowledge Survey and Assessment (KSA) project, also known as the "20-Questioner". The purpose of the KSA project is to measure the depth of technical knowledge airmen have in a wide range of technical areas. The first step of the KSA project is to transfer questions from paper-and-pencil test to a computer format and to develop an automated system of administering the tests and assessing their results. Then, over a period of several years, data will be collected from knowledge surveys that will be given to thousands of airmen. Eventually, a large enough database will have been created to be able to use an intelligent "interrogation strategy" to zero in on an airman's knowledge by carefully selecting questions. The first stage of the project, item generation (the questions of the test), was continued (from the previous summer) during this apprenticeship.

# Knowledge Survey and Assessment

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## Introduction

In evaluating a person's knowledge, the efficiency and accuracy of the results and how they can be obtained are crucial. Redundant questions would be a complete waste of time and resources, when a series of "intelligently selected" questions could give results just as accurate or even better. Because the Knowledge Survey and Assessment Project is concerned with a persons "general" knowledge, it would be illogical to give an airman a complete battery of tests from all the technical areas of interest. What is needed is a system of testing that can decide, based on the responses of the subject and a database containing the correlations of the difficulty of the items and their relation to other questions, what would be the next appropriate question to ask the examinee. The sophisticated, elaborate and extremely complex "knowledge interrogation strategy" that will be used to accomplish this can be compared to the strategy used to play the game "20 Questions."

Suppose there are 10,000 facts relevant to the field of the test. The 20-Questioner system can assume that a person's knowledge is roughly equal to that of the general public (or the average airman). After the first question, the system can begin fine-tuning the default assumptions as given by the database. Then based on correlations and question dependencies, the 20-Questioner can make an guess whether the examinee will know the answer to questions that have not been asked. For example, if an examinee does not know basic mathematics, the 20-Questioner could safely conclude that the subject probably does not know questions dealing with trigonometry, probably does not know questions having to do with physics, and it might be reasonable to assume that the examinee has very little knowledge of world history. Notice that the latter deductions do not closely correlate to the initial question as did the former, but they can generally be deducted correctly.

The possible uses for the 20-Questioner are cases in which a person's knowledge needs to be evaluated. The two most prominent applications would be in aptitude testing and adaptive training. Adaptive training presents a particular need for general knowledge assessment to be able to optimize instruction.

Methodology

The main concern in the primary stages of the 20-Questioner was to create an automated system of reading and displaying the general knowledge test questions. This will eventually become the method the 20-Questioner uses to display the question data. The Apprentice Knowledge Tests (AKT) were used to collect a sufficient amount of questions covering a broad base of technical knowledge. The AKT was previously used by airmen to bypass requirements for technical training. Recent changes in training regulations no longer allow airmen to "test out" of technical areas. Thus, the questions are not currently being used, but the possibility exists of reactivating the test in the future, so the confidentiality of the questions had to be maintained during the collection process. To combat this problem the test booklets were locked up at the end of the day along with any disk containing confidential information and any confidential information on a computers hard drive was "protected" or deleted.

The materials were acquired in a hard copy format from the actual test booklets. The booklets were scanned into a computer where an Optical Character Recognition (OCR) program converted the scanned test material into usable text. The text files were then proofread for scanning errors, which were fairly numerous due to the illustrations and inconsistencies in the format of the tests. The proofreading proved to be the most tedious task of the summer tour because sometimes it appeared as if the OCR randomly placed a tilde "~" along with various other characters in the text and confused certain text. For example, sometimes the capital letter "B" was replaced with the number eight "8". This mistake is understandable due to the similarities in shape but necessitated very careful proofreading. There were ninety-four Apprentice Knowledge Tests in all, each consisting of one hundred questions and a varying amount of illustrations.

The editing and conversion of (AKT) testing material was completed during the eight week period of the apprenticeship. Of the ninety - four test booklets seventy - five were already converted (from the previous summer) to the testing system format but still needed to be proofread. Nineteen were proofread and converted into the testing system format. Conversion to the proper testing format was long and involved but the search-and-replace option (in Borland Pascal) proved to be very useful. For example, the actual test format (pencil-and-paper test) presented answer choices in an "A,B,C,D" format while the

testing system required a "C1,C2,C3,C4" format and with a hundred questions meaning four hundred choices the quick search-and-replace option was extremely useful and saved me from hours of deleting and typing. The cut, copy and paste edit options were also very useful. Over one hundred and fifty illustrations were drawn, and approximately fifty scanned illustrations were resized and "touched up". The bitmaps (illustrations) were made to fit the screen limitations by using image attributes option in Windows Paintbrush. Large illustrations of electrical and medical diagrams posed a dilemma, but the use of a scanner and a clever way of using the Cut-and-Paste options in Windows Paintbrush and Lotus Freelance Graphics solved the problem. After the testing system, which was originally created in Microsoft Visual Basic, is converted to Delphi and the installation procedures are finalized, testing can begin and data can start being collected for the "intelligent interrogator" stage of the 20-Questioner. It will take approximately four years to complete the project, allowing for the sufficient collection of data, the building of the probability database, and the creation of the "intelligent interrogation system".

Results

All ninety-four AKT test pools have been converted, proofread, and double checked. During the term of this apprenticeship, nineteen new test pools were converted and verified, and approximately two hundred bitmaps (pictures) were drawn or touched-up.

Conclusion

The purpose of this summer research apprenticeship was to help in the development of the primary stages of the "20-Questioner" project. A few problems emerged dealing with the test pools that will have to be resolved at a later date.

1.) Apprentice Knowledge Test makers had, for various reasons decided that certain questions should be omitted, but the tests were taken out of active use before they could be updated. The questions have been marked but no firm decision has been made as to whether the questions will be used or omitted.

2.) Some of the material from the AKT pools dealt with similar specialty areas, resulting in redundant questions throughout the pools. A possible solution is to mark the question item as a duplicate, list its reappearances, and then have the administration program skip over the item if it has already asked that question during the current testing session.

3.) If the KSA project is to "measure" general knowledge (for adaptive training purposes) then additional sources of test questions will need to be added since the AKT tests contain many questions that are very Air Force specific. In addition, many of the questions are probably too difficult for use in a general knowledge test.

# THE EFFECT OF HYPERBARIC OXYGENATION AND HYPOBARIC EXPOSURE ON PERIPHERAL BLOOD MONONUCLEAR CELLS

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# The Effect of Hyperbaric Oxygenation and Hypobaric Exposure on Peripheral Blood Mononuclear Cells

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## Abstract

The proliferation of peripheral blood mononuclear cells (PBMC's) under various atmospheric stressors was studied. PBMC's were extracted from remnant blood using the density gradient provided by Histopaque solution. The cells were then exposed to conditions equivalent to 45 feet below sea level using a hyperbaric chamber, sea level as a control, and 85,000 feet above sea level using an altitude chamber. The experimental data indicated that hyperbaric oxygenation suppresses the proliferation of PBMC's, while exposure to hypobaric conditions increases cell reproduction and survival compared to the control.

# THE EFFECTS OF HYPERBARIC OXYGENATION AND HYPOBARIC EXPOSURE ON PERIPHERAL BLOOD MONONUCLEAR CELLS

Paul D. Giles

## Introduction

This experiment focuses on peripheral blood mononuclear cells (PBMC's), which are T-cells, B-cells, monocytes, and macrophages. PBMC's are a category of leukocytes, or white blood cells. Some leukocytes, such as monocytes and macrophages, are phagocytic cells with granular cytoplasm and completely segmented nuclei. A Lymphocyte is another type of leukocyte that can be a T-cell or a B-cell. These cells have nearly clear cytoplasm, simple nuclei, and are involved in antibody production. Each of these cells plays an important role in the immune function of the body; antibodies produced and regulated by lymphocytes are instrumental in the labeling of foreign particles, bacteria, and viruses for destruction by phagocytes. One function of white blood cells is the release of toxins in an area of damaged tissue in order to kill it and prevent the spread of infection. However; antibiotics, surgery, and other treatments such as hyperbaric oxygenation can kill bacteria and eliminate the need for tissue death. Therefore, decreased numbers of leukocytes in the blood of a patient with damaged tissue due to radiation, bacterial infection, or diabetic non-healing wounds can allow the tissue to be saved without endangering surrounding tissues.

Hyperbaric oxygenation (HBO) is a treatment which uses pure oxygen under pressure to maximize tissue oxygen levels. HBO is used in both emergency conditions such as decompression sickness and carbon monoxide poisoning and in clinical circumstances including osteomyelitis, radiation tissue necrosis, and compromised wounds. Many hyperbaric oxygenation patients who are treated for damaged or infected soft tissues would benefit from decreased proliferation of leukocytes in order to increase their chances of saving the tissue and decrease the need for amputation and tissue removal. Another benefit is that the occurrence of fewer lymphocytes leaves the blood system open, allowing better delivery of antibiotics to infected areas.

A measure of cell proliferation in PBMC populations when exposed to hyperbaric oxygenation shows the

effects of this treatment on the immune system. Cells exposed to the normal conditions of regular air (21% oxygen) and 1 ATM (pressure at sea-level) provide a negative control. Cells exposed to hypobaric conditions provide a positive control; this is because HBO has been shown to combat the various biological effects of high altitude exposure, as is seen in its use to treat decompression sickness ("The Bends").

Problem

The two-fold purpose of this experiment was to observe how hyperbaric oxygenation alters cell proliferation and to show that rapid decompression provides a positive control.

Methodology

To extract PBMC's from remnant blood, whole blood was diluted using phosphate buffered saline (PBS) solution. Next, in a 15 ml centrifuge tube, the blood was layered on top of a Histo-paque solution, which provided a density gradient. The tube was then centrifuged at 2,000 rpm for 15 minutes. The tube contained a top layer of blood serum with an underlying layer of PBMC's. The removed PBMC's were washed with 10 ml of PBS in a centrifuge at 800 rpm for 5 minutes. The PBMC's were then suspended in media; composed of sterile water, fetal bovine serum, penicillin, and RPMI; at a concentration between 500,000 and 1,000,000 cells per milliliter. Once the correct concentration had been reached, the cell suspension was divided into three parts and plated onto three 12 well micro-titer plates. One plate was left under sterile conditions at 1 ATM of pressure. Another plate was exposed to hyperbaric oxygenation at the equivalent depth of 45 feet below sea level (66 psi) for 90 minutes. The remaining plate was placed in an altitude chamber and exposed to the equivalent of 25,000 feet above sea level for 2 minutes. It was then rapidly decompressed to the equivalent of 85,000 feet above sea level for an additional 2 minutes. After the exposures, all three plates were kept in an incubator for three days. Cell concentrations were counted using a microscope and hemacytometer on the first and third days of incubation.

Results

Three experiments were done using initial cell concentrations of 500,000 cells/ml. After three days, cells exposed to rapid decompression showed the greatest proliferation and cells exposed to hyperbaric oxygenation showed the least proliferation in comparison to the control. Cell concentrations of cultures exposed to hyperbaric oxygenation averaged 90,000 cells/ml on the first day and 160,000 cells/ml on the third day. Control cell cultures

averaged concentrations of 130,000 cells/ml on the first day and 340,000 cells/ml on the third day. The increased cell proliferation in comparison to the negative control caused by exposure to rapid decompression shows altitude to be the positive control, as expected. The low concentrations of cells in comparison to the starting concentration can be explained by the relatively short life-time of peripheral blood cells.

Conclusion

This initial experiment has shown HBO to decrease the proliferation of leukocytes in human remnant blood. These results have potential positive ramifications for patients treated by HBO for soft tissue trauma and/or infection who would benefit from the suppression of white blood cell adhesion. However, this experiment involves only one system--a few specific types of human peripheral blood cells. This research should be repeated studying leukocytes and their relationships with surrounding cells under similar conditions, studying individual components and characteristics of peripheral blood cells under hyperbaric and hypobaric stressors, and studying the response of the human body in general. Another direction that could be taken would be to repeat the experiment using feeder cells. Feeder cells increase the life span of neutrophils by providing antioxidant protection. If the peripheral blood cells could be kept alive for longer than a few days, long term effects of varied atmospheric pressures could be better measured.

Laboratory Experience

The laboratory experience this summer has been educationally enthralling. It has given me new experiences with using laboratory equipment, such as a table-top centrifuge, a hemacytometer, a bio-safety cabinet, Eppedorf micro-pipettes, hyperbaric oxygenation chamber, and an altitude chamber. In addition to new practical knowledge of science, I increased my computer skills by working with Excel in order to make graphs of the data and follow the general trends more easily. This has been a good change from the standard lab experiments in a high school science class because it forced me to build the experiment from the ground up, and not read the step-by-step instructions out of a book.

References

Knowledge gained these past eight weeks can be attributed to intensive training and assistance from the various scientists, physicians, physiologists, and other personnel on the staff at AL/AOH, especially Dr. Edward Piepmeir and Maj. George Kemper.

# Project Critical Flicker Fusion

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# Project Critical Flicker Fusion

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**Abstract**

Critical Flicker Fusion (CFF) has been used to study neurophysiological functioning and assessment. CFF seems most sensitive to fatigue and stress. CFF was one metric used in a comprehensive study of team dynamics involving different levels of stress. Males (54) and Females (51) were assigned to mixed gender teams of 3 persons each. Data on team measures of the TIDE$^2$ study are in preparation. Prior to (1300-1400) and immediately after the TIDE$^2$ study (1630-1730), CFF measures were taken. Post test CFF scores were significantly elevated from Pre test scores. Further analysis revealed females not males had elevated Post test scores. These data suggest females are less stressed or less fatigued by the TIDE$^2$ study whereas male stress and fatigue remained constant.

# Project Critical Flicker Fusion

Cynthia O. Guerrero

## Introduction

Metrics that provide some indication of brain dynamics are important to studies of cognitive ability during stress. The Critical Flicker Fusion (CFF) technique may have strong potential in this regard. CFF has a long history of use in psychological and pharmacological research (for a review see Vaszko 1970) CFF may be a measure of visual information processing (Kerr 1992) and as such would be an important metric to studies that may be influenced by alterations in central visual processing. It is often used to assess fatigue induced by impaired sleep (Nicholson 1989). Generally, fatigue seems to decrease CFF which is usually measured in cycles per second or Hz.

CFF has a rich history in psycho physiological research. It has long been used to assess neurological functioning. For example drugs which relax (perhaps fatigue) also lower CFF (Hindmarch 1994; Manni 1993). Earlier studies related CFF to anxiety (Danjou et. al 1992; Alan, 1981) and stress (Stewart 1971). Weber (1980) found that CFF was a good measure of stress but did not induce stress.

It has been concluded in earlier research that the CFF can not be considered a sensitive indicator of general

fatigue induced by hard muscular work (Graybiel et al., 1943). In addition to fatigue, a number of factors including the use of a number of prescription drugs and other self-administered agents such as nicotine and alcohol and dysfunction of the visual system can cause a decline in CFF. Therefore, if the influence of other factors is eliminated, a decline in CFF following a tiring mental task can reasonably be attributed to mental fatigue.

Teams worked for approximately 2 1/2 hours on this computer-generated decision task, therefore it is reasonable to expect some fatigue to occur. In addition, because this is a computer task, and the CFF is a vision-based measure, it was expected that the CFF would capture vision-based fatigue. It was expected that fatigue would also be caused by the high level of stress in these situations. While the teams were able to interact with each other by sending information and text messages to other stations, they were not allowed to talk throughout the task. In addition, factors such as working under conditions of low and high time pressure, ambiguous/conflicting information and the high stakes involved contributed to the high stress level and fatigue of this simulation.

A larger study ($TIDE^2$) in which the differences in the team decision making dynamics among mixed gender teams in a US Air Force command and control decision setting were examined was conducted at Brooks AFB during the summer of 1995. The author collected data for the $TIDE^2$ study. Since

TIDE$^2$ involved varying levels of stress due to varying levels of workload and possible fatigue due to the long hours, CFF was also obtained prior to and after TIDE$^2$. It was expected that no gender differences would be found due to previous research (Shearer 1992). However, it may be that females would have a more profound decrease in CFF because of the additional stress on females in this type of situation due to the traditionally male nature of these roles. Some differences were expected in all subjects between the pre and post readings due to stress and fatigue after performance on the decision task.

## Method

### Apparatus

Critical Flicker Fusion (CFF) - The apparatus used to obtain the subjects' CFF threshold was a portable, battery-operated device made by the New Zealand Air Force Research Laboratory. The subjects looked into the device with both eyes while the intensity of a 1 x 2 cm, red, flickering light source was constant. They progressively increased the frequency by turning a dial clockwise until a change from the flickering of the light to fusion (where the light source appeared steady) was reported. This was defined as the CFF threshold which is reported in hertz (Hz).

Team Interactive Decision Exercise for Teams Incorporating Distributed Expertise (TIDE$^2$) - TIDE$^2$ was developed for the Office of Naval Research by faculty at

Michigan State University (Ilgen and Hollenbeck, 1993). This task enables the study of team decision making dynamics. The TIDE$^2$ task allows up to 4-member teams to work together through networked computers. In this study, the task was configured for 3-member teams on 80-486 Hz computers with VGA monitors.

Each subject was assigned to one of the three "stations" referred to as Alpha (team leader), Bravo, and Charlie. Their objective was to assess information of an incoming aircraft and determine what military action to take against each target based on it's threat levels. There are nine pieces of information pertaining to the threat level of each target. Each team member was trained to be a specialist on three particular pieces of information. Each station is provided with direct access to the measurements for five of the target attributes but only one of which they are trained to interpret. Each station is also able to access and receive other pieces of information from the other two stations.

Once an assessment of the information is reached, subjects choose from seven different judgments what level of action to take against the target. The levels begin at "Ignore", and progressively become more drastic through "Defend" (shoot the target down). There is also a time limit in which the decision must be reached of either 90 or 120 seconds for each target. Judgments made by Bravo and Charlie are transmitted to Alpha who then makes a final team

decision. The trial score is based on how many levels away the decision is from the correct judgment.

Subjects

There were a total of 105 subjects, including 51 females and 54 males. CFF readings taken prior to performing the TIDE$^2$ task at approximately 1:00 to 2:00 PM, and after the task at approximately 4:30 to 5:30 PM, were obtained from 105 and 95 subjects, respectively. Team scores for the TIDE$^2$ task were obtained from 81 subjects (27 teams). The majority of subjects were provided by Olsten Staffing Services and were paid for the approximately 5 hours of participation time. Also, a cash incentive to be given to the teams scoring in the top 40% was provided. All subjects were between the ages of 17 and 55 and were either taking college courses, or were enrolled to enter college.

Design

Subjects participated in teams of three people and were randomly assigned to one of the three stations. At the team level, teams were randomly assigned to one of six between-subject conditions: (a) all female, (b) all male, (c) 2 females with a female leader, (d) 2 females with a male leader, (e)2 males with a female leader, or (f) 2 males with a male leader. These were hierarchical teams in that each team had a leader who made the final decision.

Within-subject manipulations included task characteristics such as time pressure and uncertainty. That

is, every team encountered the same set of targets. The targets varied in time pressure and certainty of information. In addition several individual difference measures were collected to capture differences in personality attributes, which are described in the following section.

Procedure

Subjects were first asked to fill out questionnaires for personality traits and behavioral characteristics. These included the NEO PI-R Personality Inventory (Psychological Assessment Resources, Inc., 1992) and the FIRO B Self-scorable version (Consulting Psychologists Press, Inc., 1990). Also assessed were measures of Instrumentality/Expressiveness, and Individual versus Collectivist orientation.

Next, three readings of each subject's CFF threshold were taken, and the subject was asked to provide 3 ml of saliva to be tested for levels of testosterone and cortisol. The testosterone was collected as an additional gender-related measure, and the cortisol as an indicator of fatigue/stress.

The subjects were then randomly assigned to their stations for the TIDE$^2$ task and went through approximately 45 minutes of training for the task. The training consisted of 10 minutes reading over a handbook which explained the task, and 30 minutes of performing 6 practice trials of the

TIDE$^2$ task. The subjects then put an actigraph, or activity monitor, on their right wrist to be worn throughout the rest of the task. A subjective fatigue rating was made by each subject, and the subjects then performed 3 sets of 16 TIDE$^2$ trials. After each set, the subjects filled out questionnaires pertaining to their confidence of how well the team could score, and how efficient each individual was. At the conclusion of the session, another subjective fatigue rating was made and three readings of each subject's CFF threshold were taken.

## Results

First, the difference between scores prior to and after the task were analyzed for all subjects. The intra-subject readings were averaged, and it was determined by a t test that the change in the CFF scores was significantly different from zero ( $t=2.03$, $p < .05$). The scores, however, did not decrease, but instead increased after the task as seen in Figure 1. When this was broken down to males and females separately, the statistical technique was repeated on each one and an interaction was found between them (Figure 2). The change of increase in scores within females only was found to be significantly different from zero ($t = 2.54$, $p < .02$). However, there was no significant increase in scores prior to and after the task in males only ($t = 0.62$, $p = 0.54$).

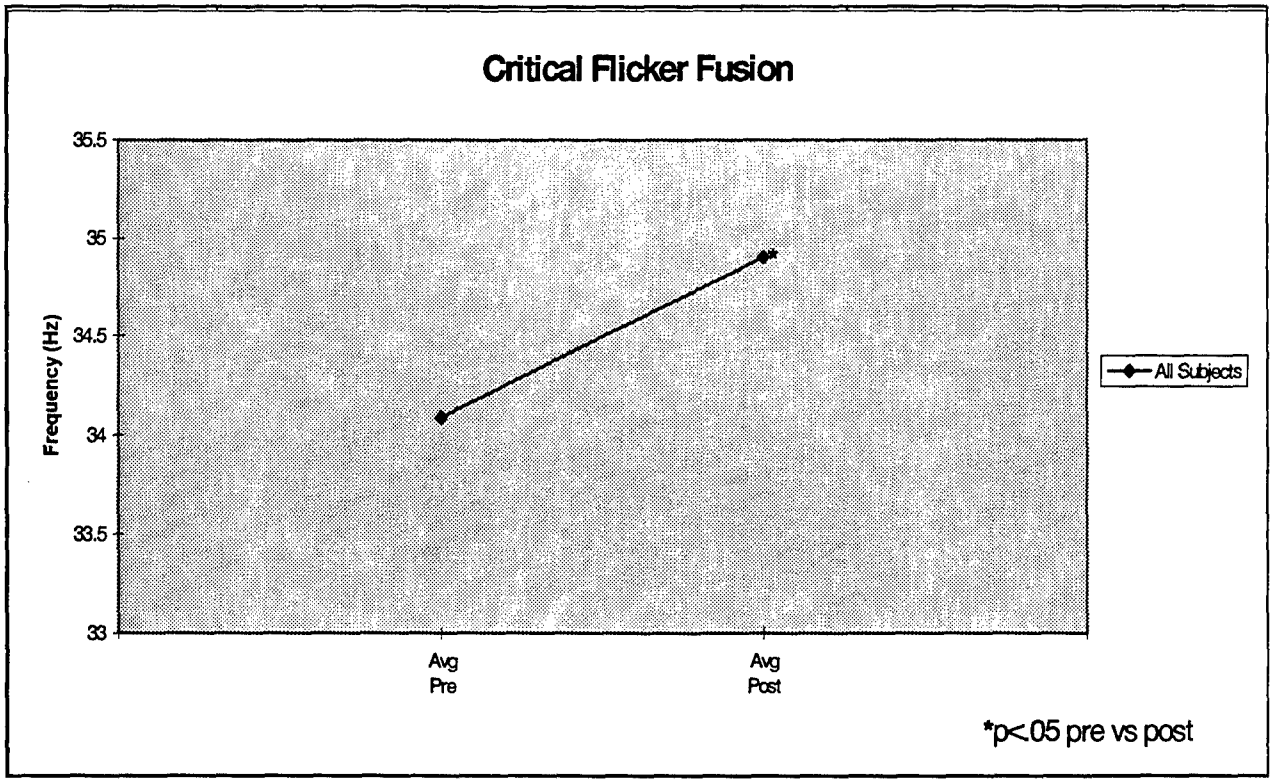


Figure 1.

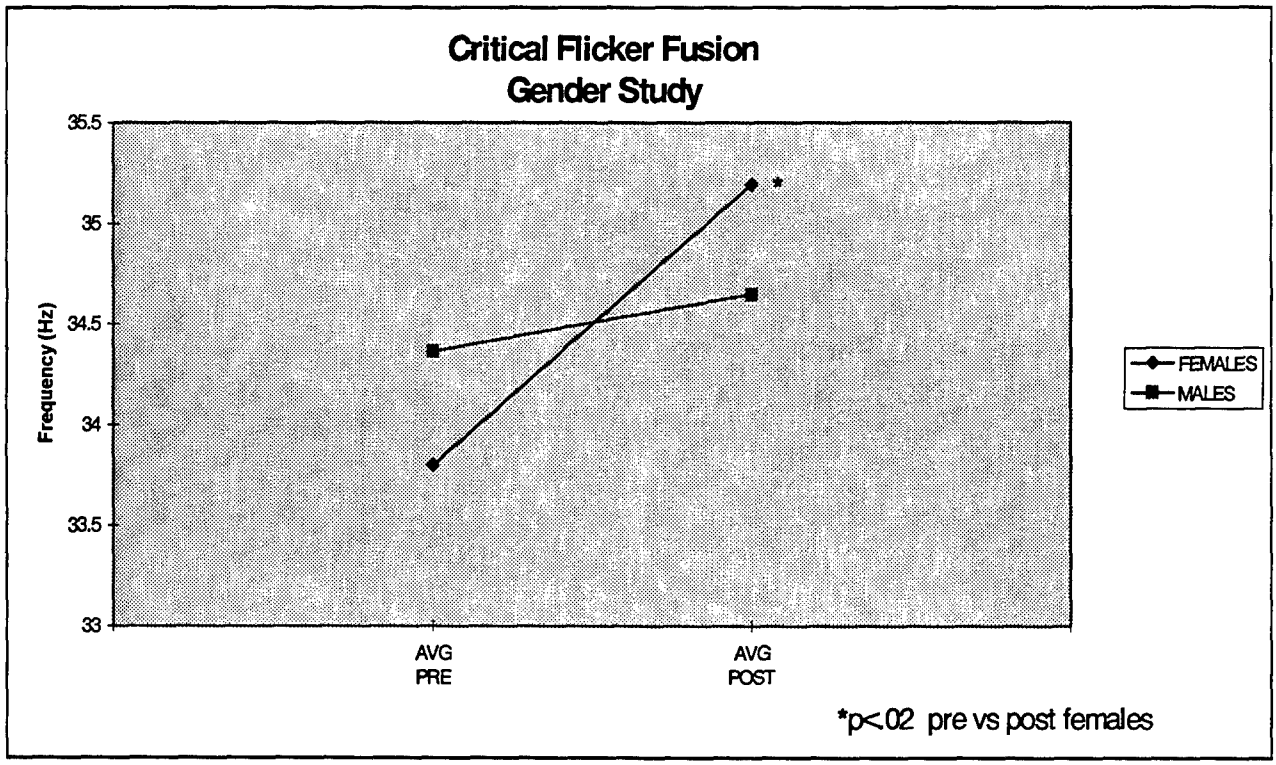


Figure 2.

## Discussion

TIDE$^2$ data are currently being evaluated and are unavailable for this study. CFF scores changed significantly after the decision task compared to readings taken prior to the task. This change was found to be greatest in females. It was predicted that females might have a more profound decrease in CFF because of the additional stress, but the opposite was found in this study. One possible reason for the elevation in CFF scores may be the body's natural circadian rhythms. There are a series of peaks and declines throughout the day in the body temperature. Alertness levels are generally higher in the late afternoon. However it is not likely these effects are circadian because the males did not show a significant rise in Post test CFF scores.

An alternative explanation for the increase in female CFF scores post test may be that females were less fatigued and/or less stressed at the end of the experiment than at the beginning. Coming into a novel environment and being asked to do a computer study of teams in a traditionally male setting may have been intimidating.

Males were not as susceptible to this before and after effect of the task on CFF. Perhaps a real gender phenomenon has been found for CFF that was missed by Shearer(1992) because of a lack of a Pre and Post test design. The subjective fatigue scores would be useful to determine if

the females experienced less fatigue at the end of the study compared to the beginning. However these are not currently available.

Gender differences were found in CFF scores that may relate to stress and fatigue. Further research on this phenomenon may be warranted, particularly in studies of gender, stress, fatigue and team dynamics.

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# HSAP Final Report

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
Bolling Air Force Base, DC

and

Armstrong Laboratory

August 1995

# HSAP Final Report

Kelly Harmon

I spent this summer working in Armstrong Laboratories at Tyndall A.F.B., Florida. I performed a few different jobs. These jobs were to do an inventory of the Technical Information Center, which is the library for government use,; to write an Eqtech; to write a Tech Payoff; and to do odd jobs for different people.

My ongoing job was to take inventory of the serials in the Technical Information Center (TIC). I was told to go to the shelves and put the issues of a serial title in chronological order, beginning with the first issue TIC has in its holdings and ending with the latest issue. After I was finished with a serial, I was to write down the holdings. Then, I went to the microfiche and wrote down the holdings TIC has of that serial title. When I finished the last serial on the shelf, I began putting the serials on the Techlib database.

Colonel Lamb asked me to do an Environmental Quality Technology Synopses (EqTech) on the skid-mounted sodium sulfide/ferrous sulfate metal treatment system. An EqTech is a one-three page illustrated flyer that describes technologies that are either complete or nearly complete. It is a publication that is distributed to military bases worldwide.

I was also asked to do a Tech Payoff on the skid-mounted sodium sulfide/ferrous sulfate metal treatment system. A Tech Payoff is a two page flyer describing a technology's benefits and background. It is also distributed to military bases worldwide.

To complete both the EqTech and the Tech Payoff I had to talk to the point of contact on this subject, Lt. Ray Smith. He gave me some information from a few different sources to use in writing these flyers. I read the information, highlighted the important parts, and wrote the flyers.

I also did odd jobs for the people who work here. I did some photocopying, cleaning an office so Major Smith could move in, putting papers and articles in 3-ring binders, and recycling. I did not mind it as long as I was kept busy.

Although I would not want to do it again, I found this summer job to be real interesting. I learned a lot about serials and the Techlib database from doing the inventory. I also participated in some hands-on computer training. We watched the training videos on Microsoft Word, Powerpoint, and Excel. Those were real interesting. I learned a lot from those. Finally, because this was my first job, I learned about work relationships and how to develop them.

# Free Radical Detection in Lyophilized Liver: An EPR Study

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
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August 1995

# FREE RADICAL DETECTION IN LYOPHILIZED LIVER: AN EPR STUDY

Brian D. Hutchens

Free Radical Detection in Lyophilized Liver: An EPR Study

## ABSTRACT

Free radical detection in lyophilized liver was studied. The purpose of the experiment was to determine the efficiency of N-tert-butyl-α phenyl nitrone, (PBN) to trap the spin label 2,2,5,5-tetramethyl -1-pyrrolidinyl oxy-3-carboxamide (3-CAR) in lyophilized liver. The samples were identified using the technique of electron paramagnetic resonance spectrometry (EPR) . The data from the EPR was then transferred via computer to WIN-EPR which analyzes the data and finds the differences in peaks. The data from the EPR was analyzed using double integrations and peak-picking to determine the difference in peaks. It was found that the trapping efficiency of PBN for the spin label 3-CAR was 31% with lyophilized mouse liver. The spin trap PBN is currently used to detect radicals in liver slices exposed to chemical carcinogens and the efficiency of the trap should be taken into account when interpreting EPR data quantitatively.

## INTRODUCTION

Understanding free radical reactions is important to the military. The main objective of this project is to study free radicals which can be detected in lyophilized tissue. A free radical is an atom, molecule or compound with one or more unpaired electrons[1]. As free radicals will attempt to gain an electron from other compounds in order to pair with their odd electrons, free radicals are classified as highly reactive. Free radicals were first postulated by Fenton in 1893. Following this early work, free radical studies expanded with the recognition that food spoilage is an oxygen free radical process.

The Armed Forces have an interest in oxidative degradation of foods because they need to be able to store food for long periods of time[2,3]. Stored food is used in space missions and by special forces deployed for short periods.

After recognition of the importance of free radicals in radiation injury, the US Army began studying ways to prevent free radical reactions[4]. Radioprotection is currently studied at the Armed Forces Institute of Radiobiology[5,6] which trains medical personnel on the effects of nuclear weapons[7]. More recently the US Army has studied radicals produced by chemical weapons and pest control agents[8-10].

Studies of free radicals are also of interest to the US Air Force for the biological effects of trichloroethylene (TCE)[11]. TCE is the best solution available to remove grease from aircraft without damaging the metal parts but because it contains chlorine it is an environmental hazard.

The US Navy Medical Research Institute studies free radicals for assessment of the damage of paints used to coat the hulls of ships to marine life. Also the Naval Research Laboratory tests the decomposition of explosives by measuring the radicals they produce[12-13].

The liver is the most important organ for metabolism of chemicals in the body. The effects of free radicals on liver can be studied using precision cut liver slices[14]. Slices can be prepared from the liver of research animals as well as from liver donors. Slices are most like the liver *in vivo* than other *in vitro* techniques because it includes all the populations of cells necessary to study cytotoxicity. The principal technique used to determine free radicals is electron paramagnetic resonance spectroscopy (EPR). EPR is the most sensitive and direct method of measuring free radicals[15]. In general the technique measures the effect of a magnetic field on an unpaired electron (free radicals and transition metals). The spinning electron acts as

a small magnet. It also interacts with neighboring nuclei. When placed in an external electric field, information is obtained regarding the local environment surrounding the unpaired electron[16] In biological systems free radicals are mostly short-lived and highly reactive species reacting at diffusion controlled rates. For this reason the technique of spin trapping is used for detection of radicals in biological tissue[17].

Spin trapping consists of reacting a short lived radical with a spin trap, usually a nitrone or nitroso compound yielding a longer lived nitroxide spin adduct which can be detected by EPR[15,17]. There are a number of spin traps which can be used to study free radicals at the cellular and sub-cellular level of tissue[15,17,18]. One of the most common traps is N-tert-butyl-α phenyl nitrone, PBN[15,17-19].

Spin labeling is a technique which makes use of stable nitroxide radicals to label biological components of a cell allowing them to be monitored by EPR[20]. A nitroxide spin label can act as a biological marker and yield information on the environment and motion of the component to which it is attached. Subtle changes in environment and motion of the spin labeled component are observed and measured through changes in the nitroxide EPR line shape. It was hypothesised that the spin label 2,2,5,5-tetramethyl -1-pyrrolidinyl oxy-3-carboxamide (3-CAR) will have a different EPR line shape when lyophilized with mouse liver slices with and without the nitroxide spin trap PBN.

PBN is currently used to trap radicals in liver slices exposed to chemical carcinogens. Understanding the efficiency of PBN to trap these radicals is important for their quantitation. The interaction of 3-CAR with PBN was used to assess the efficiency of this nitroxide to trap a free radical.

## METHODS

Chemicals

N-tert-butyl-α phenyl nitrone (PBN) and 2,2,5,5,-tetramethyl-1-pyrolidinyl oxy-3-carboxamide (3-CAR) were obtained from Aldrich and Kodak Chemical respectively. PBN (1M) was dissolved in DMSO obtained from Sigma Chemical Co, St. Louis MO.

Sample preparation

Ten slices of B6C3F1 mouse liver which were extras not needed from experiments on lipid peroxidation were used in this study. Mice which were cared for by Certified Animal Laboratory Technologists were were killed by carbon dioxide inhalation. The liver was immediately excised, cored and sliced with a tissue slicer. Each slice was homogenised in preweighed scintillation vials containing solutions supplemented with or without 10mM PBN. 3-CAR was added to the homogenate at concentrations ranging from 0 to 0.5 mM. The homogenate was frozen in liquid nitrogen and lyophilized for 18 h.

EPR analysis

The lyophilized liver homogenate was weighed. A known weight of sample was added to a glass micropipette (Clay Adams, Becton, Dickinson and Company, Parsippany, N.J.) The micropipette was placed in an EPR tube and put in the cavity of a precalibrated EMS104 EPR analyzer. The instrument parameters were: Power 25.06 mW, Sweep width 100 G, Modulation 4.02 G, Sweep Time 10.49 s, Filter time constant 20.48 ms, Receiver Gain 45 dB. The spectra were measured by peak-peak, double integration, and peak picking and compared with and without PBN. The data was normalized to liver weight. From this data the trapping efficiency of the PBN for 3-CAR was determined using the equation:

$$\text{Efficiency} = \frac{\text{EPR Reading 3-CAR}_{+\text{PBN}}}{\text{EPR Reading 3-CAR}_{-\text{PBN}}} \times 100 \qquad \text{Equation 1}$$

Data analysis

The data was analyzed by Analysis of Variance using Design Ease®, DesignEase computer program.

## RESULTS & DISCUSSION

The spin label 3-CAR is currently used to determine the concentration of radicals in samples of lyophilized liver incubated with the spin trap PBN. When the spin label 3-CAR is in solution the electrons are free to tumble giving a first derivative spectrum shown in Figure 1A.

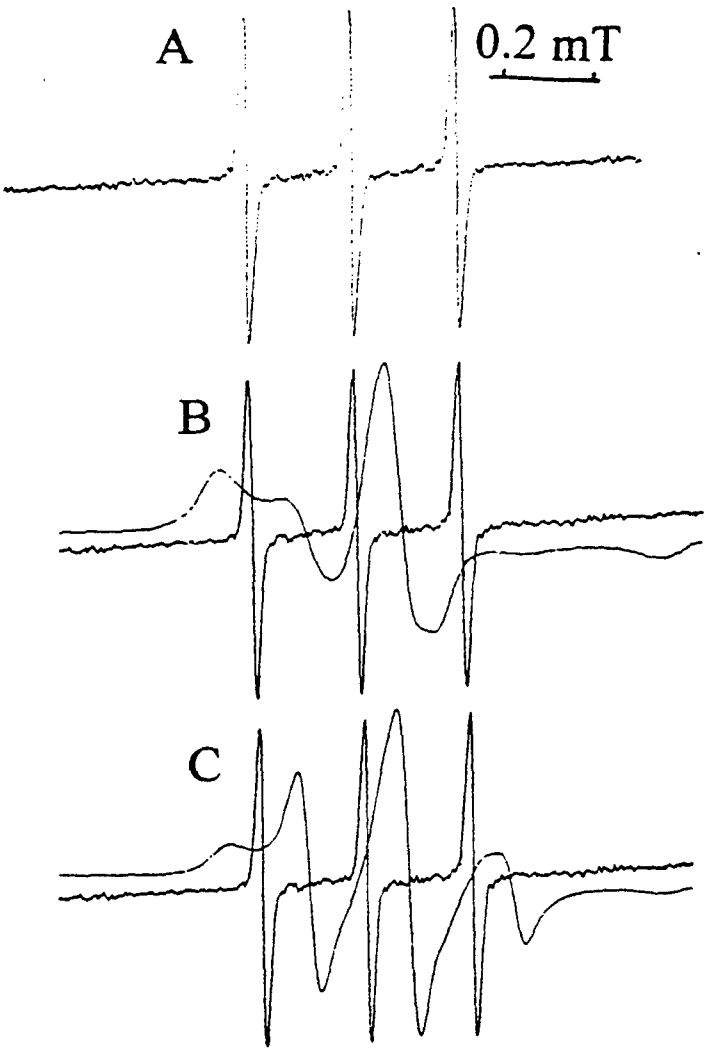


Figure 1. EPR first derivative spectra of 3-CAR. A. 3-CAR in solution B. 3-CAR lyophilized with a liver slice. C. 3-CAR lyophilized with liver slice in presence of 10 mM PBN.

Figure 1B compares this first derivative spectrum of 3-CAR in solution before and after lyophilization with liver. The hyperfine splitting of the 3-CAR spectrum is no longer the same because the electrons are immobilized. Liver will generate radicals by normal metabolism or on exposure to radical generating chemicals[15,18,20]. These radicals can be detected using the spin trap PBN. When 3-CAR is added to liver homogenate with 10 mM PBN it produces the typical first derivative spectrum shown in Figure 1C.
It is assumed that the radicals in the liver slices are the same with and without PBN. Linear regression analysis ( $y = 0.77 x - 0.97$, $r^2 = 0.86$) of the EPR arbitrary units of the peak-peak analysis of the first derivative spectra without and with PBN at each concentration of 3-CAR are shown in Figure 2.

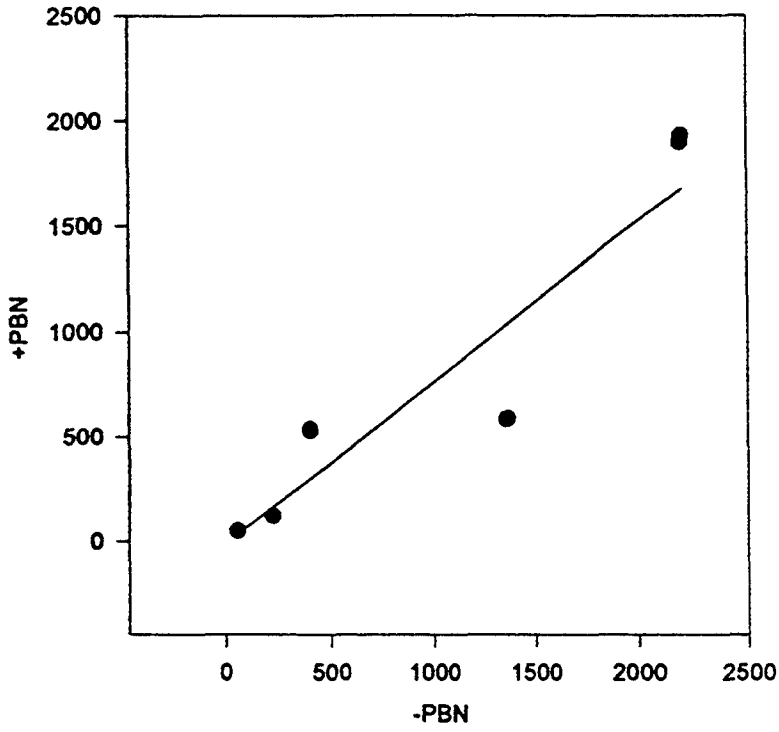


Figure 2 Linear regression analysis of peak-peak data of spectra of 3-CAR lyophilized liver without and with 10 mM PBN.

It is possible the variations from the linear response are due to the differences in the radicals in the tissues and the interaction of the trap with the 3-CAR. The latter is more probable. When the first derivative spectra of 3-CAR without and with 10 mM PBN were superimposed , Figure 3A, there were subtle differences in the hyperfine splitting between these lyophilized samples. Figure 3 B is the difference between these spectra.

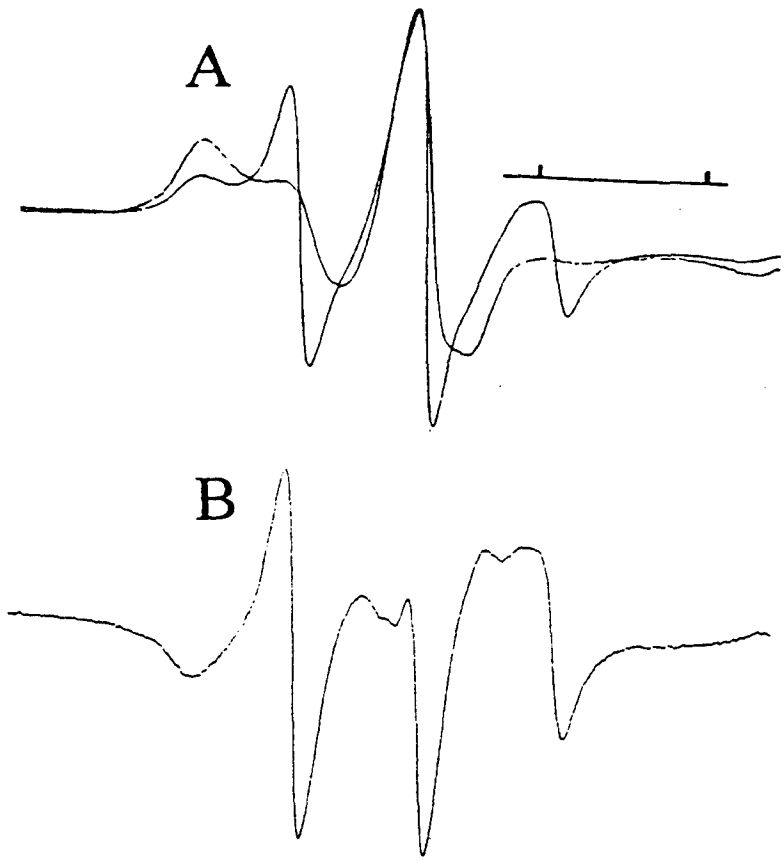


Figure 3 First derivative spectra of 3-CAR lyophilized with liver. A. Spectra 3-CAR with and without 10 MM PBN. B. Difference spectrum of first derivative spectra shown in A.

The first derivative spectra of the liver slices were computer analyzed by double integration and peak intensity at the magnetic field strength of 3481 ± 1 G, Table I. Analysis of variance of the double integration and peak height data revealed the data was normally distributed and there were significant differences between the groups ($P < 0.005$).

| Treatment Group | [PBN] mM | Double Integration EPR a.u. | Peak Height EPR a.u. |
|---|---|---|---|
| A | 0 | 1016 ± 50 | 2.71 ± 0.001 |
| A | 10 | 272 ± 22* | 1.31 ± 0.25* |
| B | 0 | 287± 3 | 1.68 ± 0.02 |
| B | 10 | 272 ± 21 | 1.09 ± 0.004* |
| C | 0 | 8374 ± 40 | 5.25 ± 0.02 |
| C | 10 | 2582 ± 103* | 3.75 ± 0.002* |
| F | 0 | 1597 ± 191 | 1.07 ± 0.001 |
| F | 10 | 972 ± 11* | 0.99 ± 0.005* |
| G | 0 | 6169 ± 356 | 3.00 ± 0.008 |
| G | 10 | 1749 ± 120* | 1.51 ± 0.02* |

Table 1 Mean ± SD of computer analysis of data., * significant difference at $P < 0.005$

Equation I was used to calculate the efficiency of the PBN to trap 3-CAR using the double integration and integration and peak height data were 33 ± 4 % and 31 ± 19 % respectively, Appendix I. Thus, using two different parameters for analysis the PBN was calculated to trap approximately 32% of the 3-CAR radicals.

Two factorial analysis of the peak height data indicated that at high concentrations of 3-CAR detection will depend on the concentration of the trap, Appendix II. PBN is a nitroxide spin trap which will react

with free radicals to form a more stable PBN adduct[15,17-20]. The concentration of PBN used in these experiments was not toxic to the liver slices. PBN is normally used as a qualitative technique[15,17-20]. To use PBN as a quantitative method it is necessary to determine its trapping efficiency. Using the spin label 3-CAR the PBN trapping efficiency was calculated to be approximately 32% of the total label present in the sample. This value is similar to the efficiency of this trap with the radical produced when TCE is irradiated (Carmichael and Steel-Goodwin unpublished data). Thus the 3-CAR radicals quantitated using the spin trap PBN represent approximately 32% of the total radicals in the sample. The efficiency of the trap should be used when interpreting quantitative radical data using the PBN spin trap.

## ACKNOWLEDGMENTS

This project is supported by the Occupational & Environmental Toxicology Division, Armstrong Laboratory, Dayton, OH and the Air Force Office of Scientific Research, Bolling AFB, Washington DC. The author performed the computer analysis of this data. He wishes to thank Dr. J. Fisher for the use of the facilities and Maj Steel-Goodwin, USAF, BSC for designing, preparing samples and writing this project.

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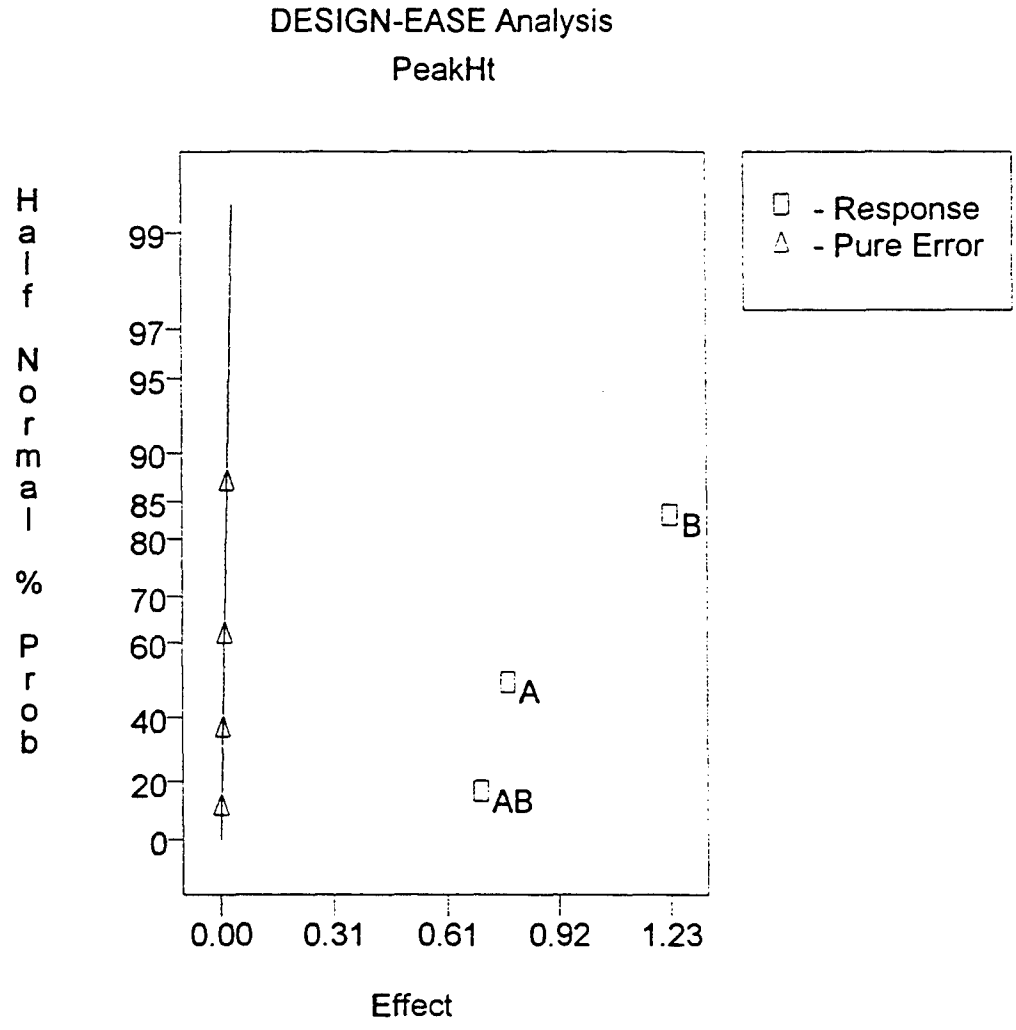
# Appendix I

## Results of calculations using Equation 1

| Double integration | Peak Height |
|---|---|
| Data | Data |
| 37.07 | 27.5 |
| 36.79 | 27.4 |
| 32.04 | 27.7 |
| 32.26 | 27.8 |
| 33.38 | 11.24 |
| 33.05 | 11.45 |
| 33.35 | 11.46 |
| 33.03 | 11.23 |
| 26.14 | 55.03 |
| 26.12 | 55.37 |
| 29.51 | 55.03 |
| 29.48 | 55.37 |
| 43.48 | 26.94 |
| 43.15 | 26.87 |
| 37.58 | 27.18 |
| 37.83 | 27.24 |
| 30.82 | 54.77 |
| 30.52 | 9.7 |
| 30.8 | 0 |
| 30.5 | 9.93 |
| 29.97 | 9.7 |
| 29.95 | 55.1 |
| 33.84 | 54.76 |
| 33.8 | 55.11 |
| 33 ± 4 | 31 ± 19 |

# Appendix II
# Two Factorial Analysis of Peak Height Data



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Analysis of PeakHt

---

| SOURCE | SUM OF SQUARES | DF | MEAN SQUARE | F VALUE | PROB > F |
|---|---|---|---|---|---|
| MODEL | 5.23948 | 3 | 1.74649 | 5010.33 | < 0.0001 |
| RESIDUAL | 0.00139 | 4 | 0.00035 | | |
| *PURE ERROR | 0.00139 | 4 | 0.00035 | | |
| COR TOTAL | 5.24088 | 7 | | | |

| | | | |
|---|---|---|---|
| ROOT MSE | 0.01867 | R-SQUARED | 1.00 |
| DEP MEAN | 1.64349 | ADJ R-SQUARED | 1.00 |
| C.V. % | 1.13601 | PRED R-SQUARED | 1.00 |

Predicted Residual Sum of Squares (PRESS) = 0.00558

* Residual = Lack-Of-Fit + Pure Error

| FACTOR | COEFFICIENT ESTIMATE | DF | STANDARD ERROR | t FOR H0 COEFFICIENT=0 | PROB > \|t\| |
|---|---|---|---|---|---|
| INTERCEPT | 1.643487 | 1 | 0.006601 | | |
| A | -0.390813 | 1 | 0.006601 | -59.21 | < 0.0001 |
| B | -0.614262 | 1 | 0.006601 | -93.06 | < 0.0001 |
| AB | 0.353388 | 1 | 0.006601 | 53.54 | < 0.0001 |

Final Equation in Terms of Coded Factors

PeakHt =

```
                1.64349
          -     0.39081 * A
          -     0.61426 * B
          +     0.35339 * A * B
```

Final Equation in Terms of Uncoded Factors

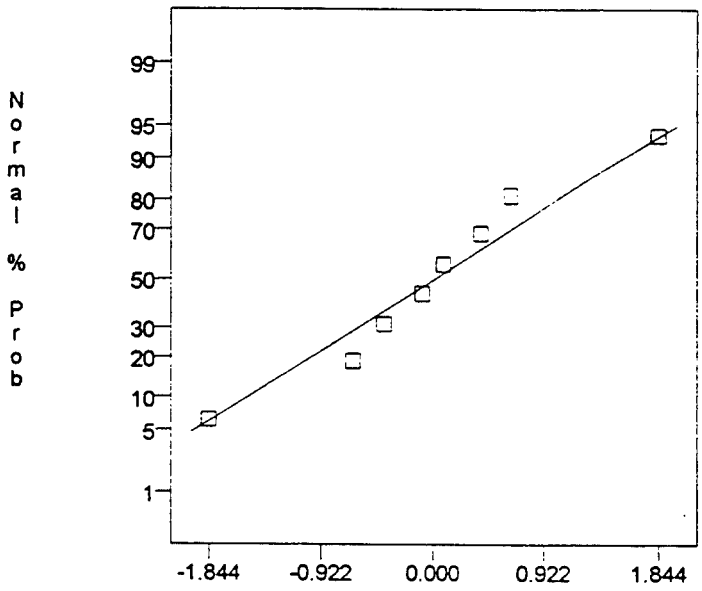
PeakHt =

```
                5.90490
          -     0.36087 * Trap
          -     9.67650 * Conc
          +     0.70678 * Trap * Conc
```

| OBS ORD | ACTUAL VALUE | PREDICTED VALUE | RESIDUAL | LEVER | STUDENT RESID | COOK'S DIST | OUTLIER T VALUE | RUN ORD |
|---|---|---|---|---|---|---|---|---|
| 1 | 2.99 | 3.00 | -0.009 | 0.500 | -0.655 | 0.107 | -0.601 | 5 |
| 2 | 3.01 | 3.00 | 0.009 | 0.500 | 0.655 | 0.107 | 0.601 | 3 |
| 3 | 1.49 | 1.51 | -0.024 | 0.500 | -1.844 | 0.850 | -4.131 | 7 |
| 4 | 1.54 | 1.51 | 0.024 | 0.500 | 1.844 | 0.850 | 4.131 | 2 |
| 5 | 1.07 | 1.07 | -0.001 | 0.500 | -0.087 | 0.002 | -0.076 | 8 |
| 6 | 1.07 | 1.07 | 0.001 | 0.500 | 0.087 | 0.002 | 0.076 | 1 |
| 7 | 0.99 | 0.99 | -0.005 | 0.500 | -0.401 | 0.040 | -0.355 | 4 |
| 8 | 1.00 | 0.99 | 0.005 | 0.500 | 0.401 | 0.040 | 0.355 | 6 |

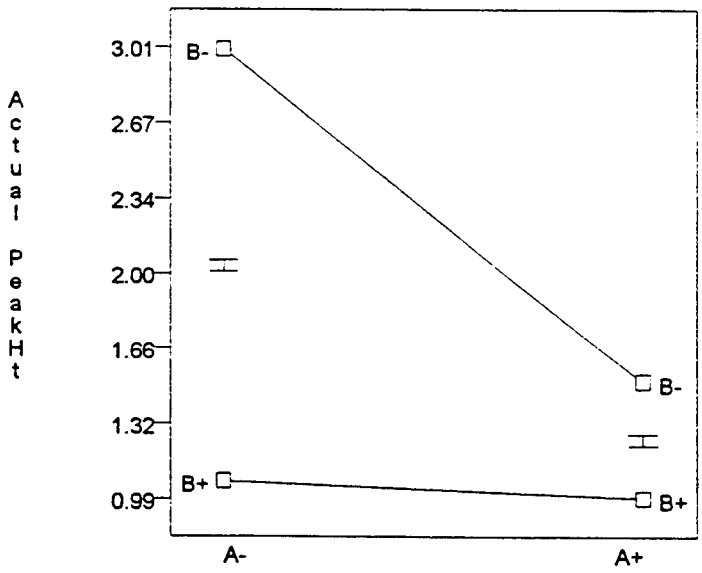
DESIGN-EASE Analysis

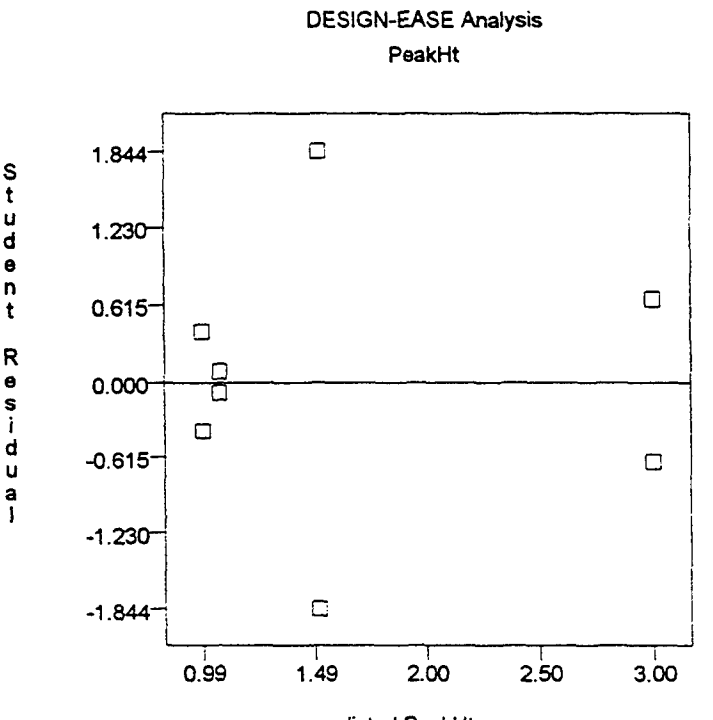
PeakHt

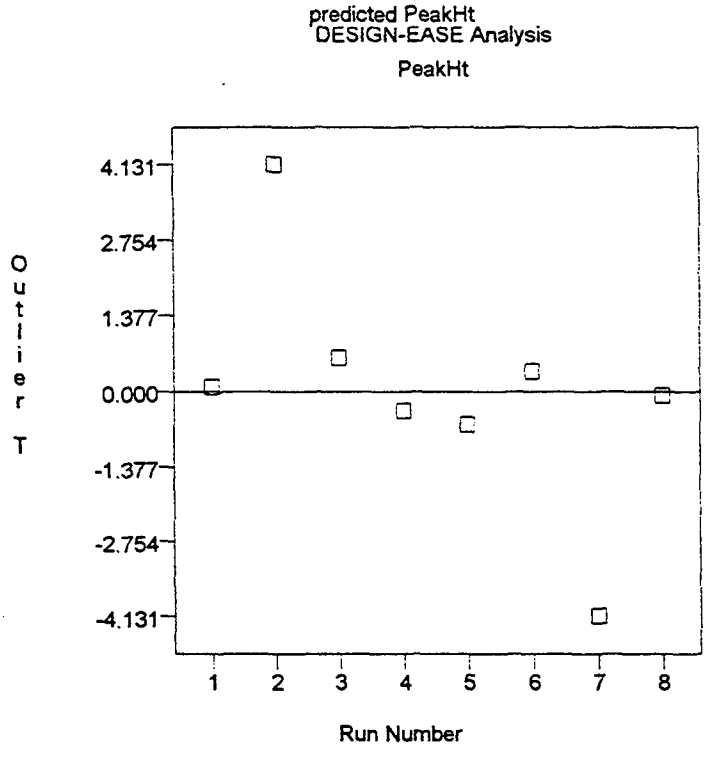



DESIGN-EASE Analysis

PeakHt




DESIGN-EASE Analysis
PeakHt
Student Residual
1.844
1.230
0.615
0.000
-0.615
-1.230
-1.844
0.99
1.49
2.00
2.50
3.00
predicted PeakHt


DESIGN-EASE Analysis
PeakHt
Outlier T
4.131
2.754
1.377
0.000
-1.377
-2.754
-4.131
1
2
3
4
5
6
7
8
Run Number